3 RESULTS AND DISCUSSION

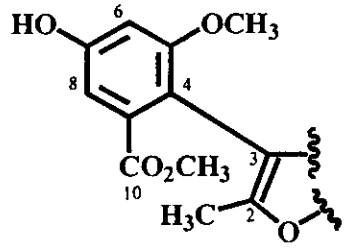
Chemical investigation of fungi was divided into two parts. The first part involved isolation, purification and structure elucidation of compounds from *Penicillium* sp. BCC 7540. Upon chromatographic separation, the broth extract yielded one new compound (**VR-JOY2**) together with four known ones (**VR-JOY3**, **VR-JOY4**, **VR-JOY5** and **VR-JOY6**). One unidentified compound (**VR-JOY1**) decomposed upon standing at room temperature. The second part dealth with the compounds isolated from two strains of *Cordyceps militaris*: BCC 2816 and BCC 2819. Chromatographic separation of the broth and mycelial extracts of *C. militaris* BCC 2816 afforded four new compounds (**VR-JOY10–VR-JOY13**) along with four known ones (**VR-JOY7–VR-JOY9** and **VR-JOY14**). Upon repeated chromatography, both broth and mycelial extracts of *C. militaris* BCC 2819 gave three known compounds (**VR-JOY7–VR-JOY9**), previously isolated from the strain BCC 2816, and one additional known compound (**VR-JOY15**). The structures were elucidated by analysis of 1D and 2D NMR spectroscopic data. The ^{13}C NMR signals were assigned from DEPT, HMQC and HMBC spectra. For known compounds, their ^{1}H and/or ^{13}C NMR data were compared with those reported in the literature.

3.1 Structure determination of compounds isolated from *Penicillium* sp. BCC 7540

3.1.1 Compound VR-JOY2

Compound **VR-JOY2** was isolated as a colorless gum. The UV spectrum (**Figure 1**) showed an absorption band due to an aromatic chromophore at λ_{max} 281 nm. Its IR spectrum (**Figure 2**) exhibited absorption bands at 3414 and 1715 cm^{-1} for a hydroxyl group and an ester carbonyl group, respectively. Its ^{1}H NMR spectrum (**Figure 3**) (**Table 46**) showed an aromatic proton at δ_H 6.64 (*d*, J = 2.5 Hz) which was coupled with an aromatic proton at δ_H 7.03 (*d*, J = 2.5 Hz) with a *meta* coupling constant, two *singlets* of methoxy protons at δ_H 3.72 and 3.71 and a *singlet* methyl

signal at δ_H 2.51. The aromatic protons were assigned to be H-6 and H-8 according to HMBC correlations (**Figure 8**) (**Table 46**). The chemical-shift value (δ_C 158.66) of C-7 suggested that the substituent was an oxygenated group. Irradiation of the aromatic proton, H-6, (**Figure 6**) enhanced the signal of the methoxyl group at δ_H 3.71, indicating that this group was located at C-5 (δ_C 157.37), *ortho* to the aromatic proton H-6, while irradiation of the other aromatic proton, H-8, (**Figure 5**) did not show NOE enhancement to any methoxy protons. In addition, both H-8 and the methoxy protons at δ_H 3.72 showed ^3J HMBC correlations with an ester carbonyl carbon (δ_C 166.75, C-10), indicating the attachment of a carbomethoxyl group at C-9 (δ_C 128.76), *ortho* to H-8. The methyl *singlet* showed HMBC correlations with an oxyquaternary carbon (δ_C 142.32, C-2) and a quaternary carbon (δ_C 113.14, C-3) which ultimately linked C-3 with the remaining carbon (δ_C 125.52, C-4) of the aromatic ring. The methyl group was assigned to be located at C-2 based on the values of carbon chemical shift for both C-2 and C-3. From these results, the structural unit A was established.



Structural unit A

Since there were only 12 signals in the ^{13}C NMR spectrum (**Figure 4**) (**Table 46**), **VR-JOY2** had a symmetrical structure and was assigned as **1**, a new furan derivative.

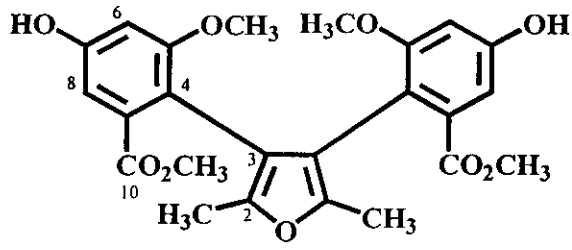


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Table 46 The NMR data of Compound **VR-JOY2**

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlation
2		142.32 (C)	
2-CH_3	2.51 (*s*)	19.10 (CH_3)	C-2, C-3
3		113.14 (C)	
4		125.52 (C)	
5		157.35 (C)	
5-OCH_3	3.71 (*s*)	56.17 (CH_3)	C-5
6	6.64 (*d*, 2.5)	103.61 (CH)	C-4, C-5, C-7, C-8
7-OH		158.66 (C)	
8	7.03 (*d*, 2.5)	108.37 (CH)	C-4, C-6, C-7, C-10
9		128.76 (C)	
10		166.75 (C=O)	
10-OCH_3	3.72 (*s*)	52.48 (CH_3)	C-10

3.1.2 Compound VR-JOY1

Compound **VR-JOY1** was isolated as a white solid. Its ^{1}H NMR spectrum (**Figure 9**) was similar to that of **VR-JOY2**. The differences were additional signals of a chelated hydroxy proton at δ_H 10.98, an aromatic proton at δ_H 6.71 (*d*, J = 0.9 Hz) and methoxy protons at δ_H 3.58. Furthermore, both *meta* aromatic protons were shifted to higher field than those of **VR-JOY2**. Upon standing at room temperature, the ^{1}H NMR spectrum showed many more signals, indicating that it was decomposed.

3.1.3 Compound VR-JOY3

Compound **VR-JOY3** was isolated as a white solid, melting at 195-196 °C. The UV spectrum (**Figure 10**) showed absorption bands due to a conjugated carbonyl chromophore at λ_{max} 272 and 308 nm. Its IR spectrum (**Figure 11**) exhibited absorption bands at 3496 (a hydroxyl group) and 1660 cm^{-1} (an ester carbonyl group). The presence of the ester carbonyl group was confirmed by a signal at δ_C 168.97 in the ^{13}C NMR spectrum (**Figure 13**) (**Table 47**). Compound **VR-JOY3** showed signals for a chelated hydroxy proton [δ_H 11.13 (*s*, 1H)], an aromatic proton [δ_H 6.56 (*s*)], two oxymethine protons [δ_H 4.65 (*dq*, *J* = 2.5 and 7.0 Hz) and 4.51 (*brs*)], two *singlets* of two methoxyl groups [δ_H 3.96 (*s*) and 3.91 (*s*)] and a methyl *doublet* at δ_H 1.58 (*d*, *J* = 7.0 Hz). The chelated hydroxyl group which was assigned to be at C-8 of the aromatic ring, *peri* position to the carbonyl carbon (δ_C 168.97), showed ^3J HMBC correlations (**Figure 15**) (**Table 47**) with an oxyquaternary carbon at δ_C 136.93 (C-7) and a quaternary carbon at δ_C 102.66 (C-8a). Moreover, the presence of only one aromatic proton at δ_H 6.56 indicated that this proton belonged to a pentasubstituted benzene ring. The aromatic proton showed ^3J cross peaks with C-7, C-8a, and an oxymethine carbon (δ_C 67.57, C-4) as well as a ^2J cross peak with an oxygenated quaternary carbon at δ_C 158.80 (C-6). These results established the location of the aromatic proton at C-5 with oxygenated substituents at C-6 and C-7 and an oxymethine substituent at C-4a. The HMBC data revealed that the methoxyl groups at δ_H 3.96 and 3.91 were attached to C-6 and C-7, respectively. The oxymethine proton at δ_H 4.51 was assigned on C-4 (δ_C 67.57) according to a HMQC cross peak (**Figure 14**). The following correlations: H-4 with C-8a, the other oxymethine proton (δ_H 4.65) with C-4 and the methyl protons (δ_H 1.58) with oxymethine carbons, C-3 (δ_C 78.26) and C-4, established the dihydroisocoumarin ring with the methyl and hydroxyl groups at C-3 and C-4, respectively. The oxymethine H-3 was vicinally coupled with the methyl protons and H-4 with coupling constant values of 7.0 and 2.5 Hz, respectively, indicating that both oxymethine protons were *cis*. Thus, **VR-JOY3** was elucidated as *cis*-3,4-dihydro-4,8-dihydroxy-6,7-dimethoxy-3-methylisocoumarin (**2**)

which was previously isolated from *Aspergillus terreus* (Arai, *et al.*, 1983). The ^{1}H NMR data of compound **VR-JOY3** were similar to those reported (**Table 47**).

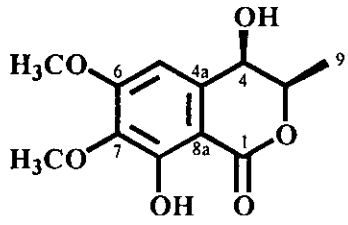

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Table 47 The NMR data of Compound **VR-JOY3** and *cis*-3,4-dihydro-4,8-dihydroxy-6,7-dimethoxy-3-methylisocoumarin

Position	VR-JOY3			isocoumarin
	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)	HMBC correlation	δ_H (*mult.*, J_{Hz})
1		168.97 (C=O)		
3	4.65 (*dq*, 2.5 and 7.0)	78.26 (CH)	C-4	4.73 (*dq*, 2.0 and 6.5)
4	4.51 (*brs*)	67.57 (CH)	C-8a	4.51 (*brs*)
4a		101.54 (C)		
5	6.56 (*s*)	102.67 (CH)	C-4, C-6, C-7, C-8a	6.75 (*s*)
6		158.80 (C)		
6-OCH_3	3.96 (*s*)	56.29 (CH_3)	C-6	3.94 (*s*)
7		136.93 (C)		
7-OCH_3	3.91 (*s*)	60.78 (CH_3)	C-7	3.76 (*s*)
8-OH	11.13 (*s*)	156.21 (C)	C-7, C-8, C-8a	11.24 (*s*)
8a		102.66 (C)		
9	1.58 (*d*, 7.0)	16.00 (CH_3)	C-3, C-4	1.49 (*d*, 6.5)

3.1.4 Compound VR-JOY4

Compound **VR-JOY4** was isolated as a white solid. Its ^{1}H NMR spectrum (**Figure 16**) (**Table 48**) showed one *singlet* aromatic proton at δ_H 6.29 which gave ^2J HMBC correlations (**Figure 19**) (**Table 48**) with two quaternary carbons at δ_C 147.90 (C-5) and δ_C 139.11 (C-7) and ^3J HMBC cross peaks with two quaternary carbons at

δ_C 112.07 (C-4) and δ_C 137.97 (C-7a). In addition, an aromatic methyl protons which appeared as a *singlet* at δ_H 2.10 exhibited ^3J cross peaks in the HMBC spectrum with C-3a (δ_C 131.80) and C-5, indicating that the aromatic methyl group was located at C-4 which was *ortho* to C-3a and C-5. From these HMBC results together with the values of carbon chemical-shift, the pentasubstituted benzene ring carrying the methyl substituent at C-4 and two oxygenated groups at C-5 and C-7 was constructed. Furthermore, the ^{1}H NMR spectrum showed a signal of an oxymethine proton at δ_H 4.45 (*dq*, J = 4.5 and 6.3 Hz, H-2) which was coupled with a methine proton at δ_H 3.04 (*dq*, J = 4.5 and 6.9 Hz, H-3) with the coupling constant value of 4.5 Hz, indicating that two methine protons had *trans* relationship (Chen, *et al.*, 2002). The splitting pattern of H-2 and H-3 as a *doublet* of *quartet*, suggested that both H-2 and H-3 were vicinally coupled with the methyl groups at δ_H 1.35 (Me-8) and δ_H 1.26 (Me-9) with the coupling constant values of 6.3 and 6.9 Hz, respectively. HMBC correlations between H-2 and C-7a and between Me-9 and C-3a established a benzodihydrofuran structure. Since no signal of other protons in the ^{1}H NMR spectrum together with the chemical-shift values of C-5 and C-7, substituents on these carbons must be hydroxyl groups. The presence of the hydroxyl groups were supported by two *broad singlets* at δ_H 4.76 and 5.22 in the ^{1}H NMR spectrum. These results indicated that **VR-JOY4** had the same structure as 2,3,4-trimethyl-5,7-dihydroxy-2,3-dihydrobenzofuran (**3**) which was previously isolated from *Penicillium citrium* F5 (Chen, *et al.*, 2002).

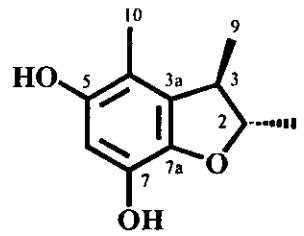

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Table 48 The NMR data of Compound **VR-JOY4** and 2,3,4-trimethyl-5,7-dihydroxy-2,3-dihydrobenzofuran

Position	VR-JOY4			2,3,4-trimethyl-5,7-dihydroxy-2,3-dihydrobenzofuran	
	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)	HMBC correlation	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)
2	4.45 (*dq*, 4.5 and 6.3)	87.58 (CH)	C-7a, C-9	4.37 (*m*)	86.70
3	3.04 (*dq*, 4.5 and 6.9)	44.45 (CH)	C-3a, C-8	3.00 (*m*)	44.30
3a		131.80 (C)			132.00
4		112.07 (C)			111.80
5-OH	5.22 (*brs*)*	147.90 (C)			149.00
6	6.29 (*s*)	102.63 (CH)	C-4, C-5, C-7, C-7a	6.20 (*s*)	102.80
7-OH	4.76 (*brs*)*	139.11 (C)			138.90
7a		137.97 (C)			138.70
8	1.35 (*d*, 6.3)	20.88 (CH_3)	C-2, C-3	1.30 (*d*, 6.5)	20.10
9	1.26 (*d*, 6.9)	19.27 (CH_3)	C-2, C-3, C-3a	1.25 (*d*, 6.5)	18.70
10	2.10 (*s*)	11.37 (CH_3)	C-3a, C-4, C-5	2.05 (*s*)	10.50

* interchangeable

3.1.5 Compound VR-JOY5

Compound **VR-JOY5** was isolated as a yellow gum. Its UV spectrum (**Figure 20**) showed an absorption band due to a conjugated carbonyl chromophore at λ_{max} 274 nm. The IR spectrum (**Figure 21**) showed absorption bands at 3370 (a hydroxyl group) and 1692 cm^{-1} (a conjugated carbonyl group). A signal at δ_C 204.14 in the ^{13}C NMR spectrum (**Figure 23**) (**Table 49**) suggested the presence of a ketone carbonyl carbon. Its ^1H NMR spectrum (**Figure 22**) (**Table 49**) showed signals of two *trans* olefinic protons [δ_H 6.82 (*qd*, J = 6.9 and 15.6 Hz) and 6.40 (*dd*, J = 1.8 and 15.6 Hz)]. The lower field olefinic proton was vicinally coupled with vinylic methyl protons at δ_H 1.95 (*dd*, J = 1.8 and 6.9 Hz) according to their multiplicity and the coupling-constant value of 6.9 Hz. The broad *singlet* (δ_H 5.98) belonged to an olefinic proton of a trisubstituted double bond. This olefinic proton gave ^3J HMBC cross peaks with an olefinic methine carbon at δ_C 125.98 (C-6), an oxymethine carbon at δ_C 76.89 (C-4) (**Figure 25**) (**Table 49**). These results established the conjugated diene

moiety. In addition, the ^{1}H NMR spectrum showed signals of two oxymethine protons at δ_H 4.80 (*d*, *J* = 2.7 Hz) and 4.23 (*d*, *J* = 2.7 Hz). The lower field oxymethine proton was assigned as H-4 according to its HMQC cross peak with C-4 (**Figure 24**). The HMBC cross peaks of H-4 with C-2, C-5 and C-6 established a cyclopentenone structure with a propenyl side chain at C-3. The location of the side chain was confirmed by ^3J HMBC data of H-6 with C-2 and C-4. The *trans* relationship between H-4 and H-5 was assigned based on the value of coupling constants of 2.7 Hz. Thus, **VR-JOY5** was identified as terrein (**4**) which was previously isolated from *Aspergillus terreus* (Cole, R. J. and Cox, R. J., 1981).

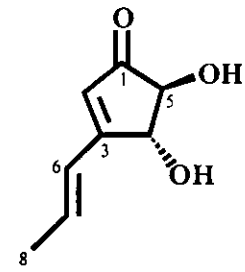

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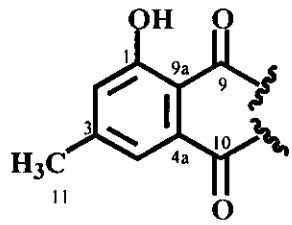
Table 49 The NMR data of Compound **VR-JOY5** and terrein

Position	VR-JOY5			terrein	
	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)	HMBC correlation	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)
1		204.14 (C=O)			203.20 (C=O)
2	5.98 (*brs*)	125.24 (CH)	C-4, C-5, C-6	5.98 (*s*)	124.50 (CH)
3		169.00 (C)			168.20 (C)
4	4.80 (*d*, 2.7)	76.89 (CH)	C-2, C-5, C-6	4.53 (*d*, 2.0)	76.30 (CH)
5	4.23 (*d*, 2.7)	81.25 (CH)	C-4	3.92 (*d*, 2.0)	80.60 (CH)
6	6.40 (*dd*, 1.8 and 15.6)	125.98 (CH)	C-2, C-4, C-8	6.34 (*d*, 17.0)	125.20 (CH)
7	6.82 (*qd*, 6.9 and 15.6)	139.85 (CH)	C-8	6.70 (*d*, 17.0)	139.10 (CH)
8	1.95 (*dd*, 1.8 and 6.9)	19.58 (CH_3)	C-6, C-7	1.95 (*d*, 0.7)	18.90 (CH_3)

3.1.6 Compound VR-JOY6

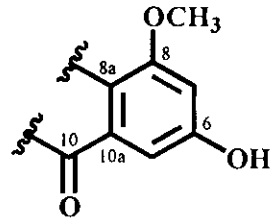
Compound **VR-JOY6** was isolated as an orange solid, melting at 225-226 °C, with a molecular formula of $C_{16}H_{12}O_5$ determined by HR-EIMS spectrum. Its UV spectrum (**Figure 26**) showed characteristic absorption bands due to an anthraquinone chromophore at λ_{max} 283, 355 and 431 nm. The IR spectrum (**Figure 27**) exhibited absorption bands of a hydroxyl group at 3456 and two carbonyl groups at 1719 and 1626 cm^{-1}. In the ^{13}C NMR spectrum (**Figure 29**) (**Table 50**), two carbonyl carbon signals at δ_C 184.51 and 186.52 were in agreement with the UV and IR data. Its ^1H NMR spectrum (**Figure 28**) (**Table 50**) showed a *singlet* signal of a chelated hydroxy proton at δ_H 13.24, two sets of *meta* aromatic protons [δ_H 7.54 (*brs*), 7.08 (*brs*), 7.30 (*d*, J = 2.5 Hz) and 6.77 (*d*, J = 2.5 Hz)], a *singlet* signal of a methoxyl group at δ_H 4.01 and a *singlet* signal of aromatic methyl protons at δ_H 2.43. The chelated hydroxy proton gave ^3J HMBC cross peaks (**Figure 33**) (**Table 50**) with an aromatic methine carbon at δ_C 124.67 (C-2) and a quaternary carbon at δ_C 115.50 (C-9a). A HMQC cross peak (**Figure 32**) between the aromatic proton at δ_H 7.08 and C-2 suggested that this proton was located at C-2, an *ortho* position of the chelated hydroxyl group. The aromatic proton at δ_H 7.54 (H-4) exhibited HMBC cross peaks with C-2, a quaternary carbon (C-9a), the carbonyl carbon (C-10, δ_C 184.51) and a methyl carbon (C-11, δ_C 21.77), indicating that the methyl and the carbonyl groups were at *ortho* position of H-4. The NOEDIFF results which gave signal enhancement of both aromatic protons after irradiation of the methyl protons (**Figure 31**), supported the purposed subunit B.



Structural unit B

One of other two *meta* aromatic protons at δ_H 7.30 (*d*, J = 2.5 Hz) was assigned to be at C-5, *peri* to the carbonyl group, according to the chemical-shift value.

Therefore, the other higher field *meta* aromatic proton was assigned as H-7. Irradiation of the methoxy protons affected only the intensity of H-7 (**Figure 30**), suggesting the location of the methoxyl group at C-8. The substituent at C-6 was then assigned as a hydroxyl group according to the chemical-shift value of C-6 (δ_C 156.23). Thus, the second structural unit C was established.



Structural unit C

Both structural units were combined to form an anthraquinone derivative. There were two possible arrangements of the structural unit C with the methoxyl group at either the upper or the lower part of the molecule. Due to HMBC correlations of both H-4 and H-5 with the same carbonyl carbon (C-10), the chelated hydroxyl and methoxyl groups were located at the same side of the molecule. Thus, **VR-JOY6** was assigned as 1,6-dihydroxy-8-methoxy-3-methylanthraquinoe (**5**) which was previously isolated from roots of *Senna lindheimeriana* (Barba, *et al.*, 1992).

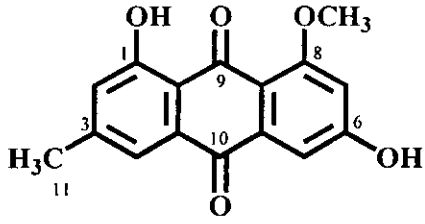


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Table 50 The NMR data of Compound **VR-JOY6**

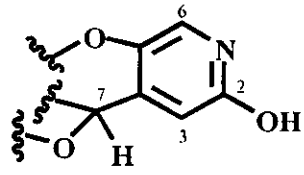
Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlation
1-OH	13.24 (*s*)	152.15 (C)	C-2, C-9a
2	7.08 (*brs*)	124.67 (CH)	C-4, C-9a, C-11
3		146.65 (C)	
4	7.54 (*brs*)	119.86 (CH)	C-2, C-9a, C-10, C-11
4a		137.23 (C)	
5	7.30 (*d*, 2.5)	107.41 (CH)	C-7, C-8a, C-10
6		156.23 (C)	
7	6.77 (*d*, 2.5)	104.81 (CH)	C-5, C-8
8		163.58 (C)	
8-OCH_3	4.01 (*s*)	56.35 (CH_3)	C-8
8a		132.29 (C)	
9		186.52 (C=O)	
9a		115.50 (C)	
10		184.51 (C=O)	
10a		113.00 (C)	
11	2.43 (*s*)	21.77 (CH_3)	C-2, C-3, C-4

3.2 Structure determination of compounds isolated from *Cordyceps militaris* BCC 2816 and BCC 2819

3.2.1 Compound VR-JOY10

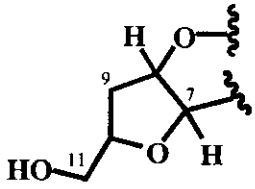
Compound **VR-JOY10** was isolated as a white solid, melting at 225-226 °C. The HR-MS exhibited a fragment ion at *m/z* 179 $(M\text{-}CH_2O)^+$ which corresponded to a $C_9H_9NO_3$ formula. Its UV spectrum (**Figure 35**) showed an absorption band due to a conjugated double-bond chromophore at λ_{max} 260 nm. Its IR spectrum (**Figure 36**) exhibited absorption bands due to hydroxyl and imine functional groups at 3225 and 1623 cm^{-1}, respectively. A signal in the ^{13}C NMR spectrum (**Figure 39**) (**Table 51**) at δ_C 157.39 confirmed the presence of the imine group. The ^1H NMR spectrum (**Figure 37**) (**Table 51**) showed two *singlet* aromatic protons at δ_H 8.42 and δ_H 8.20 which were shifted to much lower field than normal aromatic protons. The aromatic protons

gave HMQC correlations (**Figure 44**) (**Table 51**) with methine carbons at δ_C 141.14 and 153.87. Both protons gave cross peaks in the HMBC spectrum (**Figure 45**) (**Table 51**) with the same quaternary carbons at δ_C 157.39, 149.87 and 120.65. Thus, the aromatic protons at δ_H 8.42 and δ_H 8.20 were attributed to H-3 and H-6 of a 2-hydroxypyridine ring. Furthermore, H-3 showed a ^3J HMBC correlation with an oxymethine carbon (δ_C 93.57) which exhibited a HMQC correlation with H-7 [δ_H 5.95 (*d*, J = 3.0 Hz)]. These results together with the chemical-shift value of C-5 established the 2-hydroxypyridine unit D carrying an oxygenated group at C-5 and an oxymethine substituent at C-4.



Structural unit D

In the COSY spectrum (**Figure 38**), H-7 was coupled with an oxymethine proton [δ_H 4.71 (*ddd*, J = 3.0, 3.0 and 6.0 Hz, H-8)] with a coupling constant of 3.0 Hz. Furthermore, methylene protons, H_a-9 [δ_H 2.37 (*ddd*, J = 3.0, 6.0 and 12.0 Hz)] and H_b-9 [δ_H 2.05 (*ddd*, J = 6.0, 9.0 and 12.0 Hz)], were coupled with H-8 and an oxymethine proton [δ_H 4.52 (*tdd*, J = 3.0, 6.0 and 9.0 Hz, H-10)], which was further coupled with hydroxymethylene protons [δ_H 3.92 (*dd*, J = 3.0 and 12.0 Hz, H_a-11) and δ_H 3.66 (*dd*, J = 3.5 and 12.0 Hz, H_b-11)]. A HMBC correlation of H-7 with C-10 (δ_C 82.56) resulted in the formation of a tetrahydrofuran ring with an ether bridge between C-7 and C-10 (Structural unit E).



Structural unit E

Furthermore, H-7 showed ^3J cross peaks with C-5 (δ_C 149.87) and C-3 of the 2-hydroxypyridine, indicating that C-7 of unit E was connected with C-4 of the 2-hydroxypyridine unit D. Since **VR-JOY10** consisted of only four oxygen atoms, C-5 and C-8 formed an ether linkage, resulting in the formation of fused tricyclic system. This was confirmed by signal enhancement of H-3 upon irradiation of H-7 in the NOEDIFF experiment (**Figure 41**). The relative stereochemistry of the fused furan ring was *cis* since irradiation of H-8 affected the signal intensity of H-7 and H_a-9 (**Figure 42**). Enhancement of H_b-9 signal was observed when the oxymethine proton, H-10, was irradiated (**Figure 43**). This suggested that the hydroxymethyl substituent at C-10 was at the same side as fused protons, H-7 and H-8. Therefore, **VR-JOY10** was elucidated as a new 2-hydroxypyridine derivative (**6**).

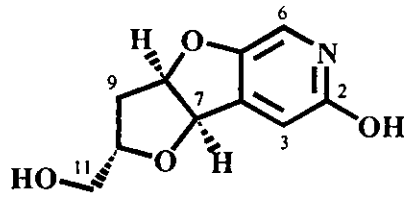


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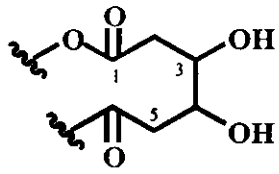
Table 51 The NMR data of Compound **VR-JOY10**

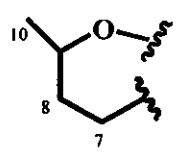
Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlation
2		157.39 (C=N)	
3	8.42 (*s*)	141.14 (CH)	C-2, C-4, C-5, C-7
4		120.65 (C)	
5		149.87 (C)	
6	8.20 (*s*)	153.87 (CH)	C-2, C-4, C-5
7	5.95 (*d*, 3.0)	93.57 (CH)	C-3, C-5, C-8, C-10
8	4.71 (*ddd*, 3.0, 3.0 and 6.0)	76.58 (CH)	
9	H_a : 2.37 (*ddd*, 3.0, 6.0 and 12.0)	34.47 (CH_2)	C-7, C-8, C-10, C-11
	H_b : 2.05 (*ddd*, 6.0, 9.0 and 12.0)		C-7, C-8, C-10, C-11
10	4.52 (*tdd*, 3.0, 6.0 and 9.0)	82.56 (CH)	
11	H_a : 3.92 (*dd*, 3.0 and 12.0)	64.17 (CH_2)	C-9, C-10
	H_b : 3.66 (*dd*, 3.0 and 12.0)		C-9

3.2.2 Compound VR-JOY9

Compound **VR-JOY9**, isolated as a colorless gum, had a molecular formula of $C_{10}H_{16}O_5$ by TOF-MS (*m/z* 216) (**Figure 56**). Its IR spectrum (**Figure 48**) showed absorption bands at 3466 (a hydroxy group), 1735 (a lactone carbonyl group) and 1728 cm^{-1} (a ketone carbonyl group). The presence of two carbonyl functionalities was confirmed by two carbon signals (**Figure 51**) (**Table 52**) at δ_C 213.94 (a ketone carbonyl carbon) and 169.62 (a lactone carbonyl carbon). The ^{13}C NMR and DEPT spectra (**Figure 51** and **52**) showed, apart from two carbonyl carbons, three methine carbons (δ_C 68.48, 72.16 and 74.34), four methylene carbons (δ_C 33.45, 39.20, 40.42 and 43.81) and one methyl carbon (δ_C 19.29). The ^1H NMR (**Figure 49**) (**Table 52**) and COSY spectra (**Figure 50**) (**Table 53**) exhibited an oxymethine proton at δ_H 4.17 (*ddd*, J = 3.0, 10.0 and 12.0 Hz, H-3) which was coupled with nonequivalent methylene protons [δ_H 2.41 (*dd*, 12.0 and 18.0 Hz, H_a-2) and 2.88 (*dd*, 3.0 and 18.0 Hz, H_b-2)] with coupling constant values of 12.0 and 3.0 Hz and with an oxymethine proton [δ_H 3.37-3.44 (*m*, H-4)] with a coupling constant of 10.0 Hz. The oxymethine proton, H-4, gave cross peaks in the COSY spectrum with nonequivalent methylene protons [δ_H 2.70 (*dd*, J = 3.0 and 18.0 Hz, H_a-5) and 4.81 (*dd*, J = 6.0 and 18.0 Hz, H_b-5)] and with the oxymethine proton, H-3. These results established a substructural unit F of which the structure was confirmed by following HMBC data (**Figure 55**) (**Table 53**). The oxymethine proton, H-3, showed ^2J HMBC correlations with the methylene carbon at δ_C 39.20 (C-2) and the oxymethine carbon at δ_C 74.34 (C-4). Furthermore, H-3 gave ^3J HMBC cross peaks with the lactone carbonyl carbon at δ_C 169.62 (C-1) and the methylene carbon at δ_C 43.81 (C-5). The oxymethine proton, H-4, showed HMBC correlations with C-3, C-5 and the ketone carbonyl carbon at δ_C 213.94 (C-6).



Structural unit F



Structural unit G

In addition, the ^{1}H NMR spectrum showed a *doublet* of a methyl group at δ_H 1.27 (*d*, *J* = 6.0 Hz, H-10) which was coupled with a methine proton [δ_H 5.04-5.15 (*m*, H-9)] in the COSY spectrum. The methine proton, H-9, was coupled with methylene protons at δ_H 2.00-2.15 (*m*, H-8) which were further coupled with methylene protons at δ_H 2.32-2.36 (*m*, H-7). In the HMQC spectrum (**Figure 54**) (**Table 52**), the oxymethine proton, H-9, showed a correlation with the oxymethine carbon at δ_C 72.16, suggesting that H-9 was attached to an oxycarbon. The second structural unit G was then purposed. HMBC correlations of H-7 and H-8 with the ketone carbonyl carbon (C-6) of the unit F, connecting C-6 of the unit F with C-7 of the unit G to form a ketone functionality. The presence of the lactone carbonyl group in the IR spectrum together with a ^3J HMBC cross peak between H-9 and C-1 suggested that the other ends of both units formed a lactone functionality. Thus, **VR-JOY9** had a cephalosporolide C skeleton (Ackland, *et al.*, 1985). The NMR data shown in **Table 52** supported this conclusion. The *trans* relationship of H-3 and H-4 was also provided by NOEDIFF results (**Figure 53**) as irradiation of the H-3 did not enhance the signal intensity of H-4. However, the relative stereochemistry of C-9 could not be assigned using NOEDIFF results. Comparison of the values of specific rotation indicated that **VR-JOY9** had all chiral centers identical to those of cephalosporolide C (7): $[\alpha]^{29}_D$ + 68° for **VR-JOY9** and $[\alpha]^{29}_D$ + 75° for cephalosporolide C.

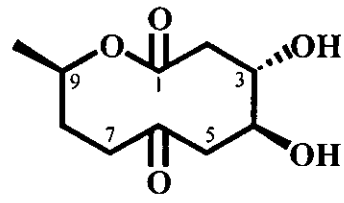


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Table 52 The NMR data of **VR-JOY9** and cephalosporolide C

Position	VR-JOY9		cephalosporolide C	
	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)
1		169.62 (C=O)		160.10 (C=O)
2	H_a : 2.41 (*dd*, 12.0 and 18.0)	39.20 (CH_2)	H_a : 2.45 (*dd*, 12.0 and 18.0)	44.10 (CH_2)
	H_b : 2.88 (*dd*, 3.0 and 18.0)		H_b : 2.93 (*dd*, 3.0 and 18.0)	
3	4.17 (*ddd*, 3.0, 10.0 and 12.0)	68.48 (CH)	4.25 (*ddd*, 3.0, 10.0 and 12.0)	69.60 (CH)
4	3.37-3.44 (*m*)	74.34 (CH)	3.45 (*m*)	75.10 (CH)
5	H_a : 2.70 (*dd*, 3.0 and 18.0)	43.81 (CH_2)	2.75 (*m*)	46.80 (CH_2)
	H_b : 2.81 (*dd*, 6.0 and 18.0)			
6		213.94 (C=O)		200.00 (C=O)
7	2.32-2.36 (*m*)	40.42 (CH_2)	not assigned	42.80 (CH_2)
8	2.00-2.15 (*m*)	33.45 (CH_2)	2.05 (*m*)	37.80 (CH_2)
9	5.04-5.15 (*m*)	72.16 (CH)	5.13 (*m*)	72.30 (CH)
10	1.27 (*d*, 6.0)	19.29 (CH_3)	1.27 (*d*, 6.5)	25.00 (CH_3

Table 53 The COSY and HMBC correlations of **VR-JOY9**

Proton	HMBC correlation	COSY correlation
H_a-2	C-1, C-3, C-4	H_b-2, H-3
H_b-2	C-1, C-3, C-4	H_a-2, H-3
H-3	C-1, C-2, C-4, C-5	H_a-2, H_b-2, H-4
H-4	C-3, C-5, C-6	H-3, H_a-5, H_b-5
H_a-5	C-3, C-4, C-6	H_b-5, H-4
H_b-5	C-3, C-4, C-6	H_a-5, H-4
H-7	C-5, C-6, C-8, C-9	H-8
H-8	C-6, C-9, C-10	H-7, H-9
H-9	C-1, C-7, C-8, C-10	H-8, H-10
Me-10	C-8, C-9	H-9

3.2.3 Compound VR-JOY12

Compound **VR-JOY12** was isolated as a colorless gum. The HRMS exhibited a fragment ion at 198 (M-CH_3OH) which corresponded to a $C_{10}H_{14}O_4$ formula. Its IR spectral data (**Figure 57**) showed the same functional group as **VR-JOY9**. The ^1H NMR signals (**Figure 58**) (**Table 54**) were similar to those of **VR-JOY9**. The minor difference was an additional signal of a methoxyl group at δ_H 3.43. An additional oxymethylcarbon at δ_C 57.38 in the ^{13}C NMR spectrum (**Figure 60**) (**Table 54**) supported the ^1H NMR data, suggesting that **VR-JOY12** was a methyl ether derivative of **VR-JOY9**. The methoxyl group was assigned to be at C-4 (δ_C 81.92) by a HMBC correlation with C-4 (**Figure 65**) (**Table 54**). The relative stereochemistry of oxymethine protons at δ_H 4.11 (*ddd*, 3.0, 9.0 and 12.0 Hz, H-3) and 3.35 (*ddd*, 3.0, 7.5 and 9.0 Hz, H-4) was assigned as *trans* by the coupling constant value of 9.0 Hz and the following NOEDIFF results. Irradiation of H-4 (**Figure 63**) did not show signal enhancement of H-3 and *vice versa* (**Figure 62**). Therefore, **VR-JOY12** was assigned to have the structure **8**, a new methyl ether derivative of cephalosporolide C with the 4-methoxyl substituent. Cross peaks in the COSY (**Figure 59**) (**Table 54**) and HMBC spectra supported the assigned structure. Since **VR-JOY12** gave the value of specific rotation of + 59°, almost identical to cephalosporolide C, all chiral centers could be assigned to be identical to those of cephalosporolide C. The CD data would be required for further analysis of configuration.

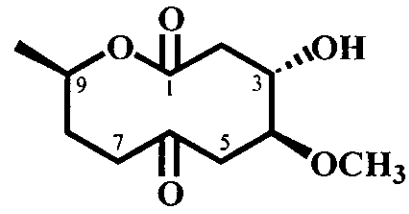

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Table 54 The NMR data of Compound **VR-JOY12**

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlation	COSY correlation
1		169.19 (C=O)		
2	H_a : 2.44 (*dd*, 12.0 and 18.0)	39.74 (CH_2)	C-1, C-3, C-4	H_b-2, H-3
	H_b : 2.86 (*dd*, 3.0 and 18.0)		C-1, C-3, C-4	H_a-2, H-3
3	4.11 (*ddd*, 3.0, 9.0 and 12.0)	68.28 (CH)		H_a-2, H_b-2, H-4
4	3.35 (*ddd*, 3.0, 7.5 and 9.0)	81.92 (CH)	C-3, C-6, 4-OCH_3	H-3, H_a-5, H_b-5
4-OCH_3	3.43 (*s*)	57.38 (CH_3)	C-4	
5	H_a : 2.63 (*dd*, 3.0 and 18.0)	41.69 (CH_2)	C-3, C-4, C-6	H-4, H_b-5
	H_b : 2.93 (*dd*, 7.5 and 18.0)		C-3, C-4, C-6	H-4, H_a-5
6		208.56 (C=O)		
7	H_a : 2.42 (*ddd*, 3.5, 6.5 and 13.5)	40.35 (CH_2)	C-6	H_b-7, H_a-8, H_b-8
	H_b : 2.33 (*ddd*, 3.5, 11.0 and 13.5)			H_a-7, H_a-8, H_b-8
8	H_a : 2.07-2.16 (*m*)	33.15 (CH_2)	C-6	H_a-7, H_b-7, H_b-8, H-9
	H_b : 1.98-2.06 (*m*)			H_a-7, H_b-7, H_a-8, H-9
9	5.05-5.12 (*m*)	71.64 (CH)		H_a-8, H_b-8, H-10
10	1.25 (*d*, 6.0)	19.64 (CH_3)	C-8, C-9	H-9

3.2.4 Compound VR-JOY13

Compound **VR-JOY13** isolated as a colorless gum, gave the same molecular formula as **VR-JOY12**. Its IR (**Figure 67**) spectral data were similar to those of **VR-JOY12**. In addition, the ^{1}H NMR (**Figures 58** and **68**) and ^{13}C NMR (**Figures 60** and **70**) spectra of **VR-JOY12** and **VR-JOY13** were alike. The location of the methoxyl group was found to be identical to **VR-JOY12** according to the HMBC data (**Figure 74**) (**Table 55**). The NOE enhancement (**Figure 72**) of the oxymethine proton at δ_H 4.28 (*ddd*, 3.0, 3.0 and 11.0 Hz, H-3) was observed after irradiation of the other oxymethine proton at δ_H 3.98 (*ddd*, 3.0, 4.5 and 11.0 Hz, H-4), suggesting that they were *cis* relationship. The coupling constant value of 3.0 Hz supported the relative stereochemistry. From these results together with COSY (**Figure 69**) (**Table 55**) and HMBC data, **VR-JOY13** had the structure **9**, a new methyl ether derivative of cephalosporolide C of which the structure differed from that of **VR-JOY12** in the

configuration at C-4. Since all ten-membered lactones isolated from the crude extract had the same configurations at chiral C-3 and C-9, it was assumed at this stage that **VR-JOY13** differed from **VR-JOY12** in the configuration of C-4 (**9**). This should be further proved by CD analysis.

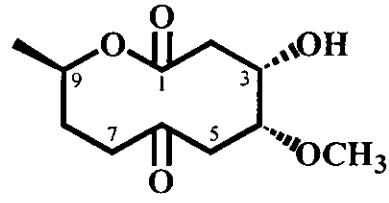

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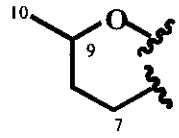
Table 55 The NMR data of Compound **VR-JOY13**

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlation	COSY correlation
1		169.86 (C=O)		
2	H_a : 2.70 (*dd*, 3.0 and 18.0)	37.43 (CH_2)	C-1, C-3, C-4	H_b-2, H-3
	H_b : 2.57 (*dd*, 11.0 and 18.0)		C-1, C-3, C-4	H_a-2, H-3
3	4.28 (*ddd*, 3.0, 3.0 and 11.0)	66.68 (CH)		H_a-2, H_b-2, H-4
4	3.98 (*ddd*, 3.0, 4.5 and 11.0)	78.84 (CH)	C-3	H-3, H_a-5, H_b-5
4-OCH_3	3.41 (*s*)	57.37 (CH_3)	C-4	
5	H_a : 2.81 (*dd*, 4.5 and 18.0)	42.89 (CH_2)	C-3, C-4, C-6	H-4, H_b-5
	H_b : 2.51 (*dd*, 11.0 and 18.0)		C-3, C-4, C-6	H-4, H_a-5
6		209.72 (C=O)		
7	H_a : 2.30 (*ddd*, 3.0, 7.5 and 13.0)	40.49 (CH_2)	C-6, C-8, C-9	H_b-7, H_a-8, H_b-8
	H_b : 2.41 (*ddd*, 3.0, 11.0 and 13.0)		C-6, C-8, C-9	H_a-7, H_a-8, H_b-8
8	H_a : 1.96-2.02 (*m*)	33.98 (CH_2)	C-6, C-7, C-9	H_a-7, H_b-7, H_b-8, H-9
	H_b : 2.05-2.13 (*m*)		C-6, C-7, C-9	H_a-7, H_b-7, H_a-8, H-9
9	5.00-5.07 (*m*)	71.96 (CH)		H_a-8, H_b-8, H-10
10	1.23 (*d*, 6.0)	19.51 (CH_3)	C-8, C-9	H-9

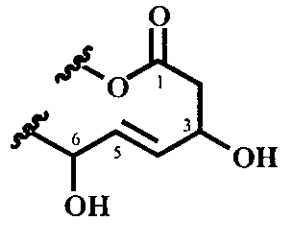
3.2.5 Compound VR-JOY11

Compound **VR-JOY11** was isolated as a white solid, melting at 203-204 °C. The IR spectrum (**Figure 76**) showed absorption bands at 3448 (a hydroxyl group)

and 1720 cm^{-1} (a lactone carbonyl group). The ^{13}C NMR spectrum (**Figure 79**) (**Table 56**) showed ten carbon signals; one lactone carbonyl carbon (δ_C 170.25), two olefinic methine carbons (δ_C 130.31 and 132.97), three oxymethine carbons (δ_C 66.78, 74.25 and 72.87), three methylene carbons (δ_C 31.34, 36.99 and 43.98) and one methyl carbon (δ_C 20.60). These suggested that **VR-JOY11** might be a ten-membered lactone ring with one double bond, two hydroxyl groups and one secondary methyl group. The ^1H NMR spectrum (**Figure 77**) (**Table 56**) showed signals of the methyl group [δ_H 1.14 (*d*, J = 6.0 Hz)], three methylene groups [δ_H 2.48 (*dd*, J = 3.6 and 12.0 Hz), 2.53 (*dd*, J = 3.9 and 12.0 Hz), 1.62-1.70 (*m*), 1.91-2.00 (*m*), 1.53-1.61 (*m*) and 1.71-1.82 (*m*)], three oxymethine protons [δ_H 4.61-4.65 (*m*), 4.08-4.15 (*m*) and 4.72-4.82 (*m*)] and two olefinic protons [δ_H 5.76 (*dd*, J = 3.0 and 16.0 Hz) and 5.63 (*ddd*, J = 1.2, 8.1 and 16.0 Hz)]. The COSY spectrum (**Figure 78**) (**Table 56**) indicated that **VR-JOY11** was composed of the same structural unit G as found in **VR-JOY9**. The configuration of the double bond was *trans* as two olefinic protons were coupled with the coupling constant value of 16.0 Hz. From the COSY spectrum and their splitting pattern, each olefinic proton was further coupled with an oxymethine proton (δ_H 4.61-4.65 or δ_H 4.08-4.15). The oxymethine protons at δ_H 4.61-4.65 and δ_H 4.08-4.15 were both assigned as allylic protons, H-3 and H-6, respectively, based on ^3J HMBC correlations (**Figure 81**) (**Table 56**). Furthermore, two separated methylene protons [δ_H 2.48 (*dd*, J = 3.6 and 12.0 Hz), 2.53 (*dd*, J = 3.9 and 12.0 Hz)] gave HMBC cross peaks with the lactone carbonyl carbon (δ_C 170.25, C-1), an oxymethine carbon (δ_C 66.78, C-3) and an olefinic carbon (δ_C 132.97, C-4). The structural unit H was then established. The connection of the structural units G and H to form a ten-membered lactone was achieved based on HMBC correlations between H-7/C-5 and H-9/C-1. The configuration of all chiral centers was obtained by X-ray analysis (**Figure 83**). Therefore, **VR-JOY11** was a new (3*S*,6*R*,9R)-decarestrictine C_2 (**10**) of which the structure differed from the synthetic (3*R*,6*S*,9*R*)-decarestrictine C_2 (Arai, *et al.*, 2000) in the configuration at C-6.



Structural unit G



Structural unit H

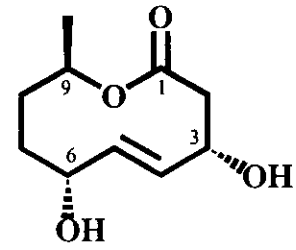


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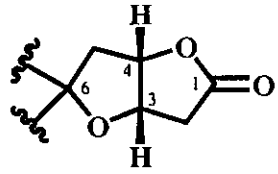
Table 56 The NMR data of **VR-JOY11**

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlation	COSY correlation
1		170.25 (C=O)		
2	2.48 (*dd*, 3.6 and 12.0)	43.98 (CH_2)	C-1, C-3, C-4	H-3
	2.53 (*dd*, 3.9 and 12.0)		C-1, C-3, C-4	H-3
3	4.61-4.65 (*m*)	66.78 (CH)	C-2, C-4, C-5	H-2, H-4
4	5.76 (*dd*, 3.0 and 16.0)	132.97 (CH)	C-3, C-5	H-3, H-5
5	5.63 (*ddd*, 1.2, 8.1 and 16.0)	130.31 (CH)	C-3, C-4	H-4, H-6
6	4.08-4.15 (*m*)	74.25 (CH)	C-4	H-5, H-7
7	1.62-1.70 (*m*)	36.99 (CH_2)	C-5, C-8, C-9	H-6, H-8
	1.91-2.00 (*m*)		C-5, C-8, C-9	H-6, H-8
8	1.53-1.61 (*m*)	31.34 (CH_2)	C-7, C-9	H-7, H-9
	1.71-1.82 (*m*)		C-7, C-9	H-7, H-9
9	4.72-4.82 (*m*)	72.87 (CH)	C-1, C-7	H-8, H-10
10	1.14 (*d*, 6.0)	20.60 (CH_3)	C-8, C-9	H-9

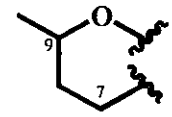
3.2.6 Compound VR-JOY7

Compound **VR-JOY7** was isolated as a white solid, melting at 92-93 °C. The molecular ion at *m/z* 198 in the EIMS spectrum (**Figure 92**) corresponded to a molecular formula of $C_{10}H_{14}O_4$. Its IR absorption band (**Figure 84**) at 1771 cm^{-1} indicated that **VR-JOY7** had a γ-lactone functionality (Ackland, *et al.*, 1985). The presence of the γ-lactone carbonyl group was confirmed by a carbon signal at δ_C 177.50 (C-1) in the ^{13}C NMR spectrum (**Figure 87**) (**Table 57**). The ^1H NMR spectrum (**Figure 85**) (**Table 57**) showed a signal of an oxymethine proton at δ_H 5.24

(*dd*, J = 6.0 and 6.0 Hz, H-4) which was coupled with an oxymethine proton at δ_H 4.91 (*dd*, J = 6.0 and 7.8 Hz, H-3). The COSY spectrum (**Figure 86**) demonstrated that H-3 was coupled only with H_a-2 [δ_H 2.85 (*dd*, J = 7.8 and 18.6 Hz)] with the coupling constant value of 7.8 Hz, but not with H_b-2 [δ_H 2.50 (*d*, J = 18.6 Hz)], while H-4 was coupled with only H_a-5 (δ_H 2.21, *dd*, J = 6.0 and 14.1 Hz) with the coupling constant value of 6.0 Hz, but not with H_b-5 (δ_H 2.33, *d*, J = 14.1 Hz). Both coupled oxymethine protons, H-3 and H-4, gave cross peaks with the lactone carbonyl carbon (C-1) and a dioxyquaternary carbon at δ_C 115.25 (C-6). The fused bicyclic structure unit I was then established based on above HMBC data (**Figure 91**) (**Table 58**) together with the lack of a hydroxyl absorption band in the IR spectrum. Irradiation of H-4 enhanced the signal of H-3 in the NOEDIFF spectrum (**Figure 89**), suggesting that they were *cis* relationship.



Structural unit I



Structural unit G

The ^{1}H NMR spectrum also showed signals for protons of the structural unit G: methyl protons [δ_H 1.16 (*d*, J = 6.0 Hz, H-10)], an oxymethine proton [δ_H 4.10-4.20 (*m*, H-9)] and four methylene protons [δ_H 1.38-1.45 (*m*, H_a-8), 1.85-1.92 (*m*, H_b-8), 2.16-2.09 (*m*, H_a-7) and 1.98-2.08 (*m*, H_b-7)]. HMBC cross peaks of H-7/C-6 and H-8/C-6 joined both units to form a spirolactone. Comparison of the ^{1}H and ^{13}C spectral data with those of cephalosporolide E (**11**) indicated that **VR-JOY7** was cephalosporolide E which was previously isolated from *Cephalosporium aphidicola* (Ackland, *et al.*, 1985).

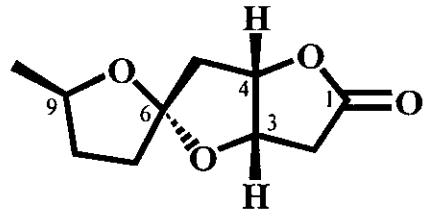


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Table 57 The NMR data of **VR-JOY7** and cephalosporolide E

Position	**VR-JOY7**		cephalosporolide E	
	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)
1		177.50 (C=O)		175.60 (C=O)
2	H_a : 2.85 (*dd*, 7.8 and 18.6)	37.28 (CH_2)	H_a : 2.67 (*dd*, 8.0 and 19.0)	41.40 (CH_2)
	H_b : 2.50 (*d*, 18.6)		H_b : 2.52 (*dd*, 1.5 and 19.0)	
3	4.91 (*dd*, 6.0 and 7.8)	77.71 (CH)	4.82 (*ddd*, 1.5, 6.0 and 8.0)	77.10 (CH)
4	5.24 (*dd*, 6.0 and 6.0)	84.21 (CH)	5.09 (*dd*, 6.0 and 6.0)	83.10 (CH)
5	H_a : 2.21 (*dd*, 6.0 and 14.1)	41.07 (CH_2)	H_a : 2.21 (*dd*, 6.0 and 14.0)	37.30 (CH_2)
	H_b : 2.33 (*d*, 14.1)		H_b : 2.33 (*d*, 14.0)	
6		115.25 (C)		114.90 (C)
7	H_a : 2.09-2.16 (*m*)	33.80 (CH_2)	1.99 (*m*)	34.00 (CH_2)
	H_b : 1.98-2.08 (*m*)			
8	H_a : 1.38-1.45 (*m*)	30.97 (CH_2)	1.33 (*m*)	31.10 (CH_2)
	H_b : 1.85-1.92 (*m*)		2.00 (*m*)	
9	4.10-4.20 (*m*)	74.84 (CH)	4.10 (*m*)	74.80 (CH)
10	1.16 (*d*, 6.0)	19.90 (CH_3)	1.10 (*d*, 7.0)	20.70 (CH_3)

Table 58 The HMBC correlations of **VR-JOY7** and **VR-JOY8**

Proton	**VR-JOY7**	**VR-JOY8**
H_a-2	C-1, C-3, C-4	C-1, C-3, C-4
H_b-2	C-1, C-3, C-4	C-1, C-4
H-3	C-1, C-4, C-6	C-1, C-2, C-4
H-4	C-1, C-6	C-1, C-3, C-6
H_a-5	C-3, C-4, C-6, C-7	C-3, C-4, C-6, C-7
H_b-5	C-3, C-4, C-6, C-7	C-3, C-6, C-7

Table 58 (Continued)

Proton	VR-JOY7	VR-JOY8
H_a-7	C-5, C-6, C-8, C-9	C-5, C-6, C-8, C-9
H_b-7	C-5, C-6, C-8, C-9	C-5, C-6, C-8, C-9
H_a-8	C-6, C-7, C-9, C-10	C-6, C-7, C-9, C-10
H_b-8	C-6, C-7, C-9, C-10	C-6, C-7, C-9, C-10
H-9	C-7	C-6
Me-10	C-8, C-9	C-8, C-9

3.2.7 Compound VR-JOY8

Compound **VR-JOY8** was isolated as a colorless gum. The mass spectrum (**Figure 100**) indicated that **VR-JOY8** had the same molecular formula as **VR-JOY7**. Its IR spectrum (**Figure 93**) was almost identical to those of **VR-JOY7**. Their ^{13}C NMR spectra (**Figure 96**) (**Table 59**) were alike except for the slightly difference in chemical-shift values. In addition, the ^{1}H NMR spectrum (**Figure 94**) (**Table 59**) was also similar to that of **VR-JOY7**, suggesting that **VR-JOY8** might be an isomer of **VR-JOY7**. Comparision of the ^{1}H and ^{13}C NMR data with those previously reported indicated that **VR-JOY8** was cephalosporolide F (**12**), the isomer of cephalosporolide E (**11**).

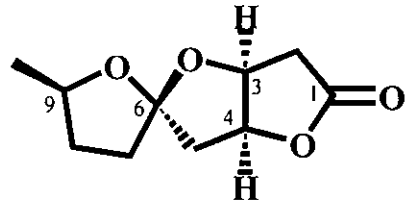

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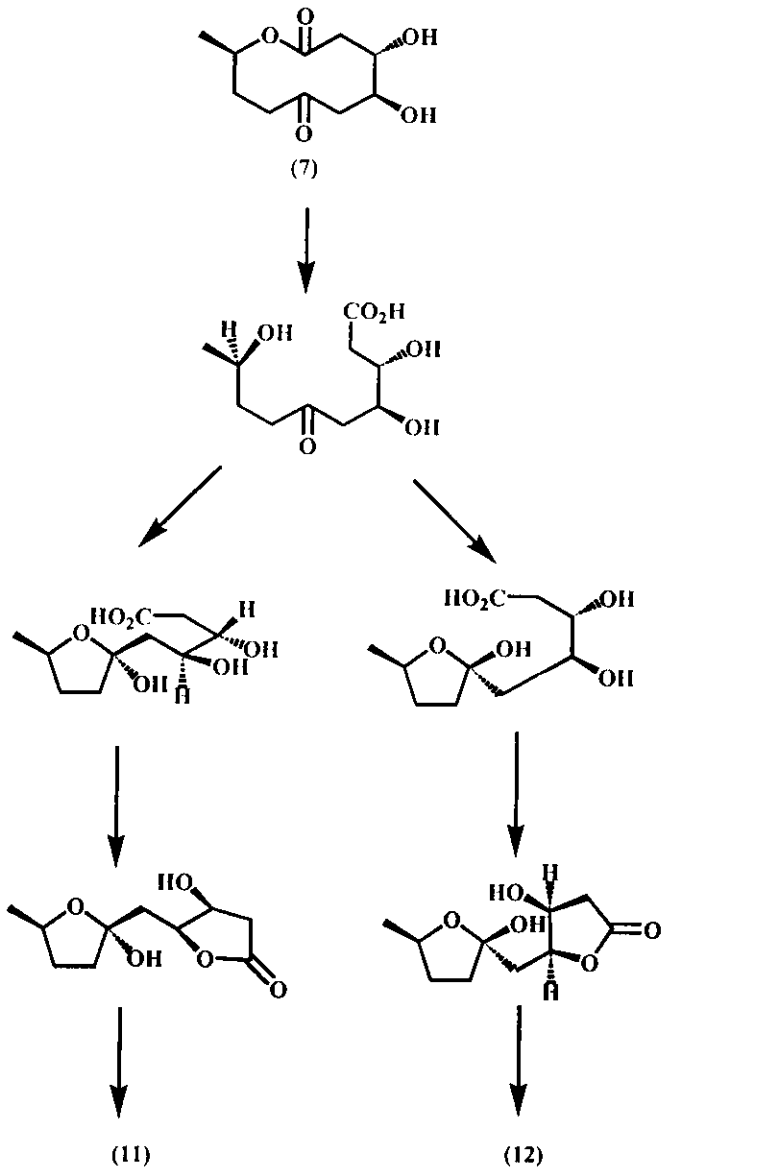
Table 59 The NMR data of **VR-JOY8** and cephalosporolide F

Position	VR-JOY8		cephalosporolide F	
	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)	δ_H (*mult.*, J_{Hz})	δ_C (C-Type)
1		175.75 (C=O)		175.50 (C=O)
2	H_a : 2.65 (*d*, 18.3)	35.91 (CH_2)	H_a : 2.68 (*dd*, 0.5 and 18.0)	42.00 (CH_2)
	H_b : 2.70 (*dd*, 5.7 and 18.3)		H_b : 2.73 (*dd*, 6.0 and 18.0)	
3	4.72 (*dd*, 4.5 and 5.7)	76.52 (CH)	4.75 (*ddd*, 0.5, 5.0 and 6.0)	76.90 (CH)
4	5.03 (*ddd*, 2.1, 4.5 and 6.6)	83.79 (CH)	5.05 (*ddd*, 2.0, 5.0 and 7.0)	83.60 (CH)
5	H_a : 2.25 (*dd*, 2.1 and 15.0)	42.00 (CH_2)	H_a : 2.27 (*dd*, 2.0 and 15.0)	36.70 (CH_2)
	H_b : 2.44 (*dd*, 6.6 and 15.0)		H_b : 2.46 (*dd*, 7.0 and 15.0)	
6		115.41 (C)		115.30 (C)
7	H_a : 2.02-2.10 (*m*)	36.86 (CH_2)	2.05 (*m*)	35.80 (CH_2)
	H_b : 1.88-1.93 (*m*)			
8	H_a : 1.60-1.71 (*m*)	32.31 (CH_2)	H_a : 1.67 (*m*)	32.20 (CH_2)
	H_b : 1.88-2.01 (*m*)		H_b : 2.05 (*m*)	
9	4.07-4.18 (*m*)	76.76 (CH)	4.15 (*m*)	76.50 (CH)
10	1.20 (*d*, 6.0)	22.68 (CH_3)	1.23 (*d*, 6.0)	22.60 (CH_3)

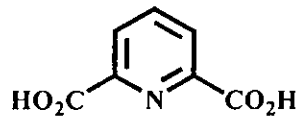
Surprisingly, the ^{1}H NMR spectra of both **VR-JOY7** and **VR-JOY8**, after standing at room temperature in a solution of $CDCl_3$, gave prominent signals belonging to a mixture of **VR-JOY7** and **VR-JOY8**. These results indicated that **VR-JOY8** is transformed to **VR-JOY7** and *vice versa* by opening the tetrahydrofuran ring at ether linkage and then rotating to form a new ether bond. Formation of **VR-JOY7** and **VR-JOY8** was shown in **scheme 2**. It was suggested that they might be derived from cephalosporolide C (7) by hydrolysis, cyclization and subsequent acetal formation and were artifacts of the isolation procedure (Ackland, *et al.*, 1985). As the ^{1}H NMR signals of both compounds appeared in the crude extract of *Cordyceps militaris*, they were fungal metabolites, not the artifacts in our case.



Scheme 2 Formation of cephalosporolides E (**11**) and F (**12**) from cephalosporolide C (7)

3.2.8 Compound VR-JOY14

Compound **VR-JOY14** was isolated as a white solid, melting at 228-229 °C. Its IR spectrum (**Figure 102**) showed absorption bands at 2800-3400 (a hydroxyl group of a carboxylic acid) and 1706 cm^{-1} (a carbonyl group of a carboxylic acid). The ^{1}H NMR spectrum (**Figure 103**) showed signals of three *ortho* coupled aromatic protons at δ_H 8.34 (*d*, *J* = 7.8 Hz, 2H) and at δ_H 8.19 (*t*, *J* = 7.8 Hz, 1H). In addition, ^{13}C NMR spectrum (**Figure 104**) showed four signals at δ_C 165.86, 147.47, 139.41 and 127.64. Thus, **VR-JOY14** was identified as pyridinedicarboxylic acid (**13**).



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3.2.9 Compound VR-JOY15

Compound **VR-JOY15** was isolated as a yellow gum. Its UV spectrum (**Figure 105**) showed an absorption band due to a chromophore of a conjugated double bond at λ_{max} 268 nm. The IR spectrum (**Figure 106**) exhibited absorption bands at 3400-2900 (a hydroxyl group of a carboxylic acid) and 1713 cm^{-1} (a carbonyl group of a carboxylic acid group). The presence of the carboxylic carbonyl functionality was confirmed by a signal at δ_C 175.37 in the ^{13}C NMR spectrum (**Figure 109**) (**Table 60**). Furthermore, the ^{13}C NMR signals showed four sp^2 carbons at δ_C 154.81 (C-6), 147.96 (C-3), 107.40 (C-4) and 105.15 (C-5). The ^{1}H NMR spectrum (**Figure 107**) (**Table 60**) showed an aromatic proton at δ_H 6.05 (*d*, *J* = 3.0 Hz, H-4) which was coupled with the other aromatic proton at δ_H 5.92 (*d*, *J* = 3.0 Hz, H-5) with the coupling constant value of 3.0 Hz. These results indicated that **VR-JOY15** was a 2,5-disubstituted furan. The aromatic proton, H-4, showed ^{3}J HMBC correlation (**Figure 111**) (**Table 60**) with C-2 (δ_C 35.00) of which methylene protons [δ_H 3.53 (*s*, H-2)] showed ^{2}J HMBC correlations with the oxyquaternary C-3 and the

carboxylic carbonyl carbon C-1, indicating that the substituent at C-2 was a carboxylmethyl group. In addition, the ^{1}H NMR spectrum showed signals of 3-hydroxybutyl side chain: $CH_3CHOH\text{-}CH_2CH_2$- [δ_H 3.83 (*sextet*, J = 6.0 Hz, H-9), 2.65-2.70 (*m*, H-7), 1.69-1.78 (*m*, H-8) and 1.18 (*d*, J = 6.0 Hz)] which was located at C-6 according to the HMBC data of H-7/C-5 and H-8/C-6. Thus, **VR-JOY15** had the known structure (**14**) which was previously isolated from *Cordyceps militaris* and synthesized (Suzuki, *et al.*, 1995).

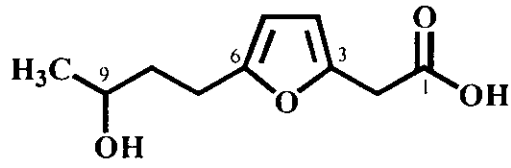

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Table 60 The NMR data of **VR-JOY15**

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlation
1		175.37 (C=O)	
2	3.53 (*s*)	35.00 (CH_2)	C-1, C-3, C-4
3		147.96 (C)	
4	6.05 (*d*, 3.0)	107.40 (CH)	C-2, C-3, C-5, C-6
5	5.92 (*d*, 3.0)	105.15 (CH)	C-3, C-4, C-C-6
6		154.81 (C)	
7	2.65-2.70 (*m*)	23.93 (CH_2)	C-5, C-6, C-8, C-9
8	1.69-1.78 (*m*)	37.04 (CH_2)	C-6, C-9, C-10
9	3.83 (*sextet*, 6.0)	66.37 (CH)	C-7, C-8, C-10
10	1.18 (*d*, 6.0)	22.02 (CH_3)	C-8, C-9

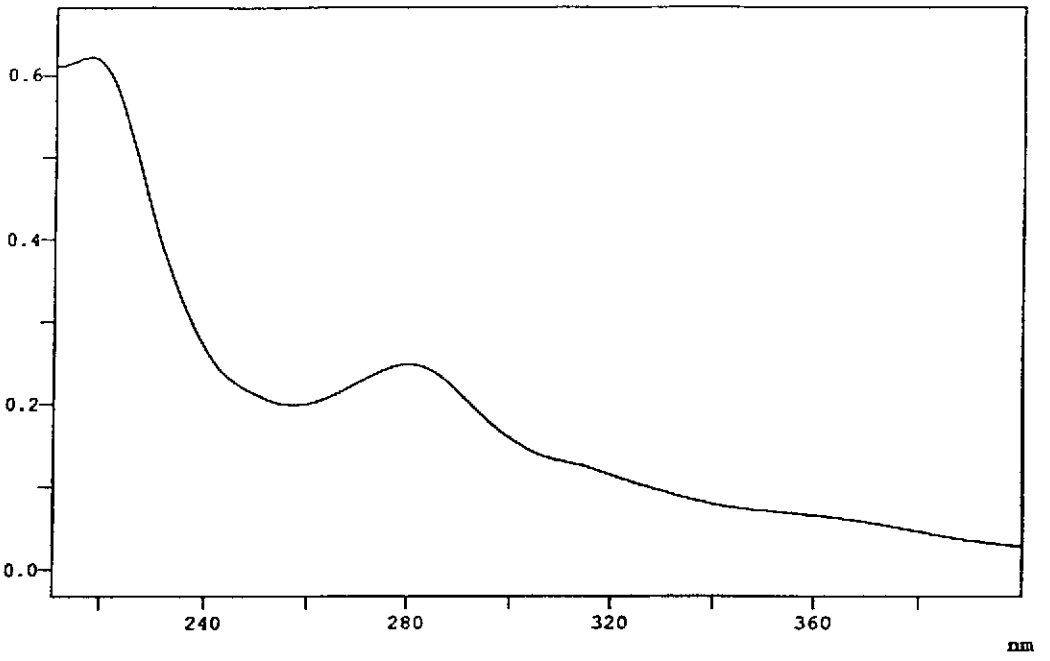


Figure 1 UV (MeOH) spectrum of VR-JOY2

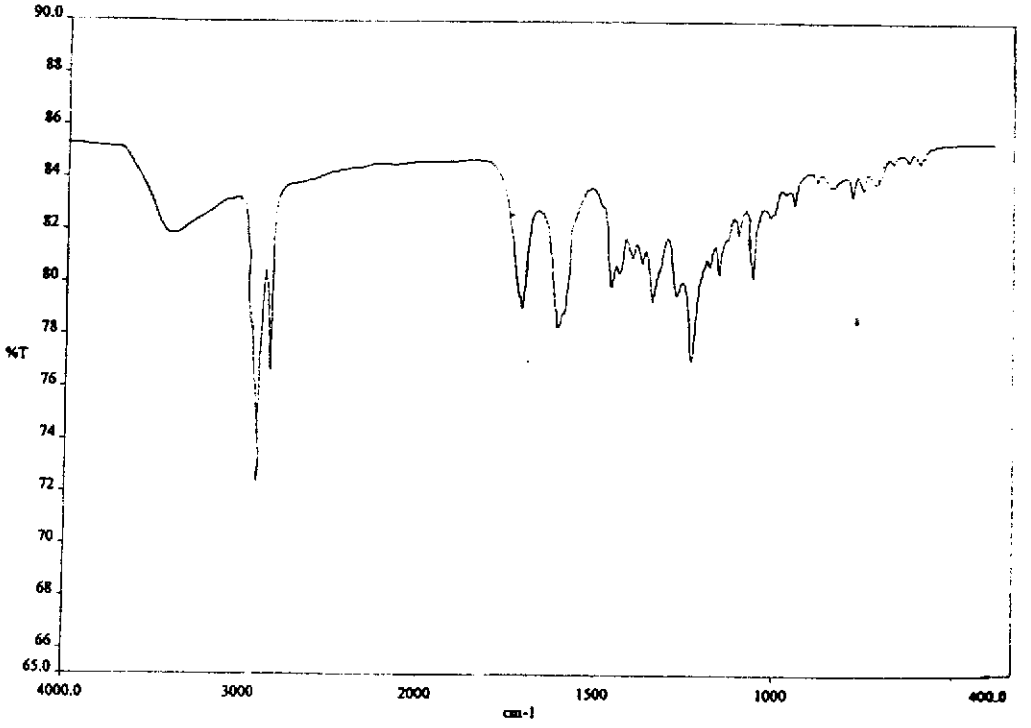


Figure 2 FT-IR (neat) spectrum of VR-JOY2

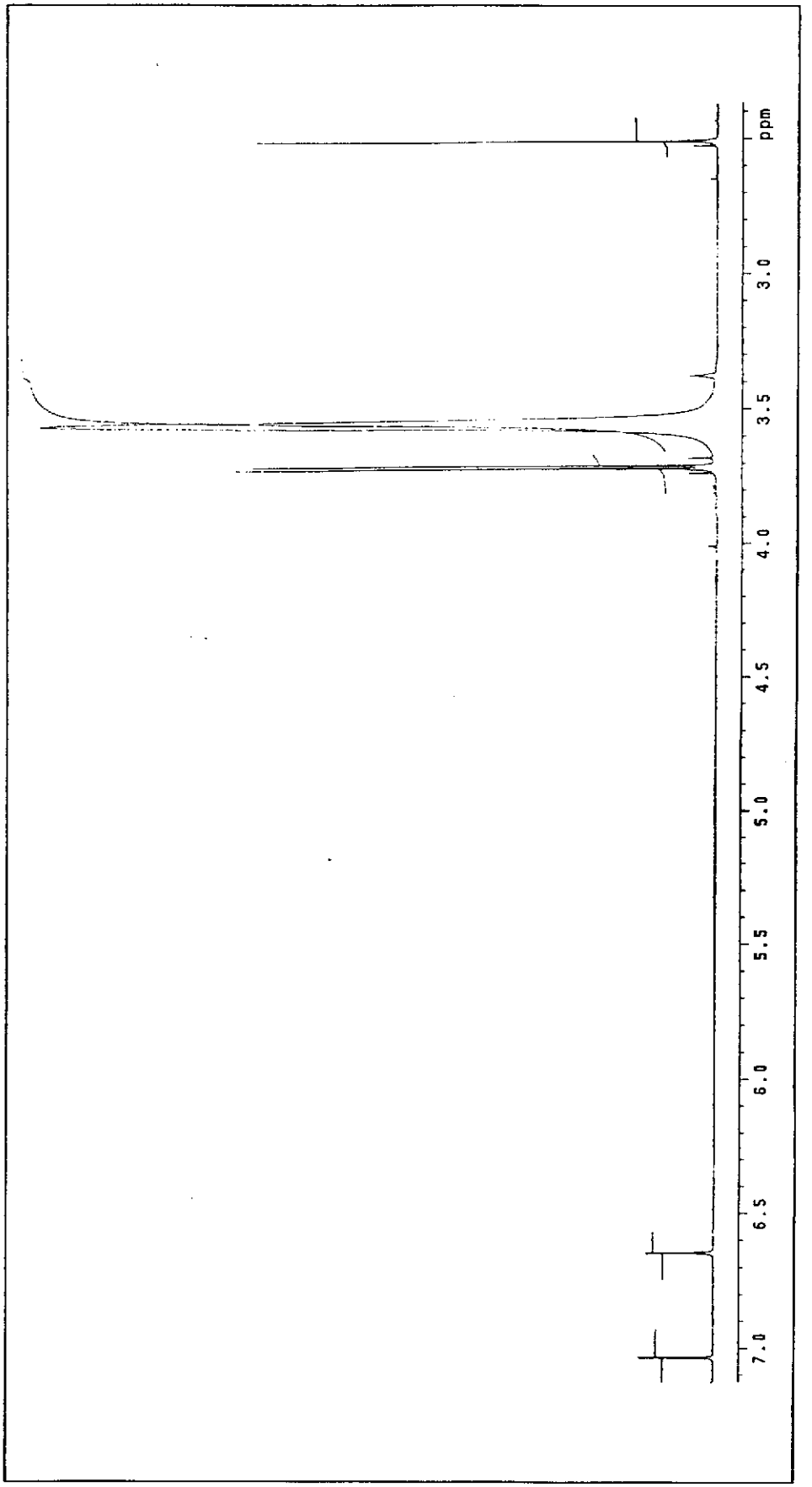


Figure 3 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of VR-JOY2

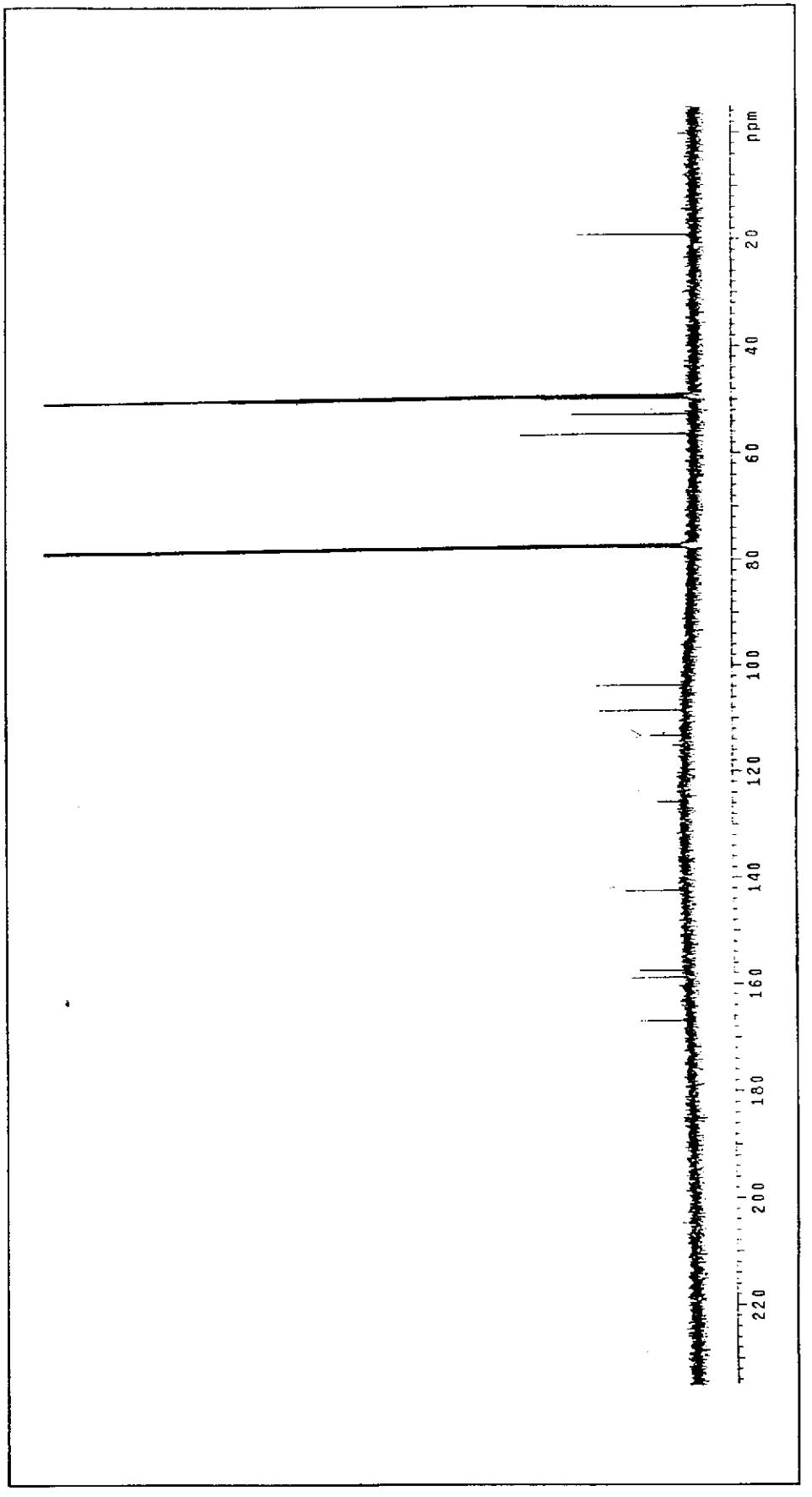


Figure 4 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of VR-JOY2

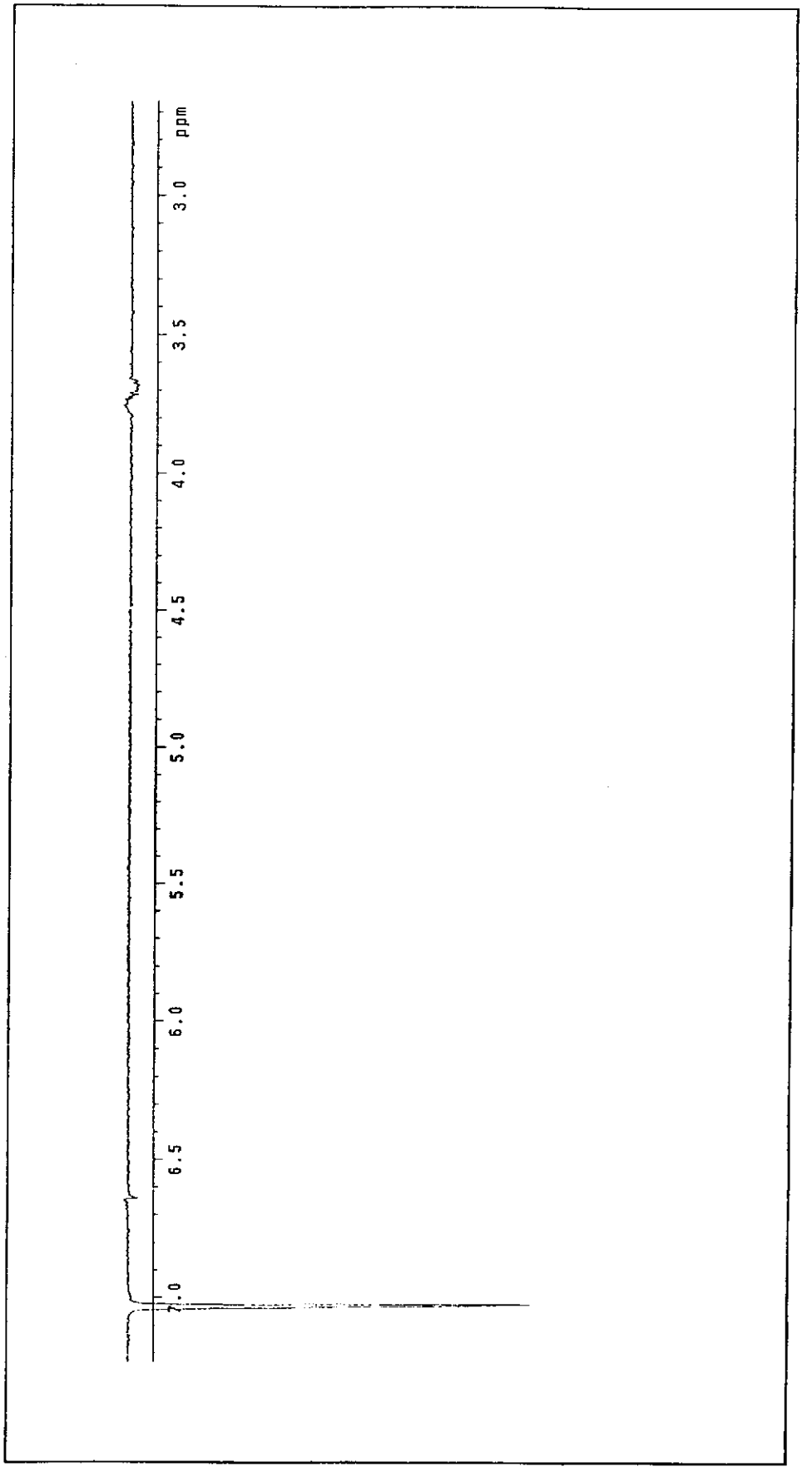


Figure 5 NOEDIFF spectrum of VR-JOY2 after irradiation at δ_H 7.03

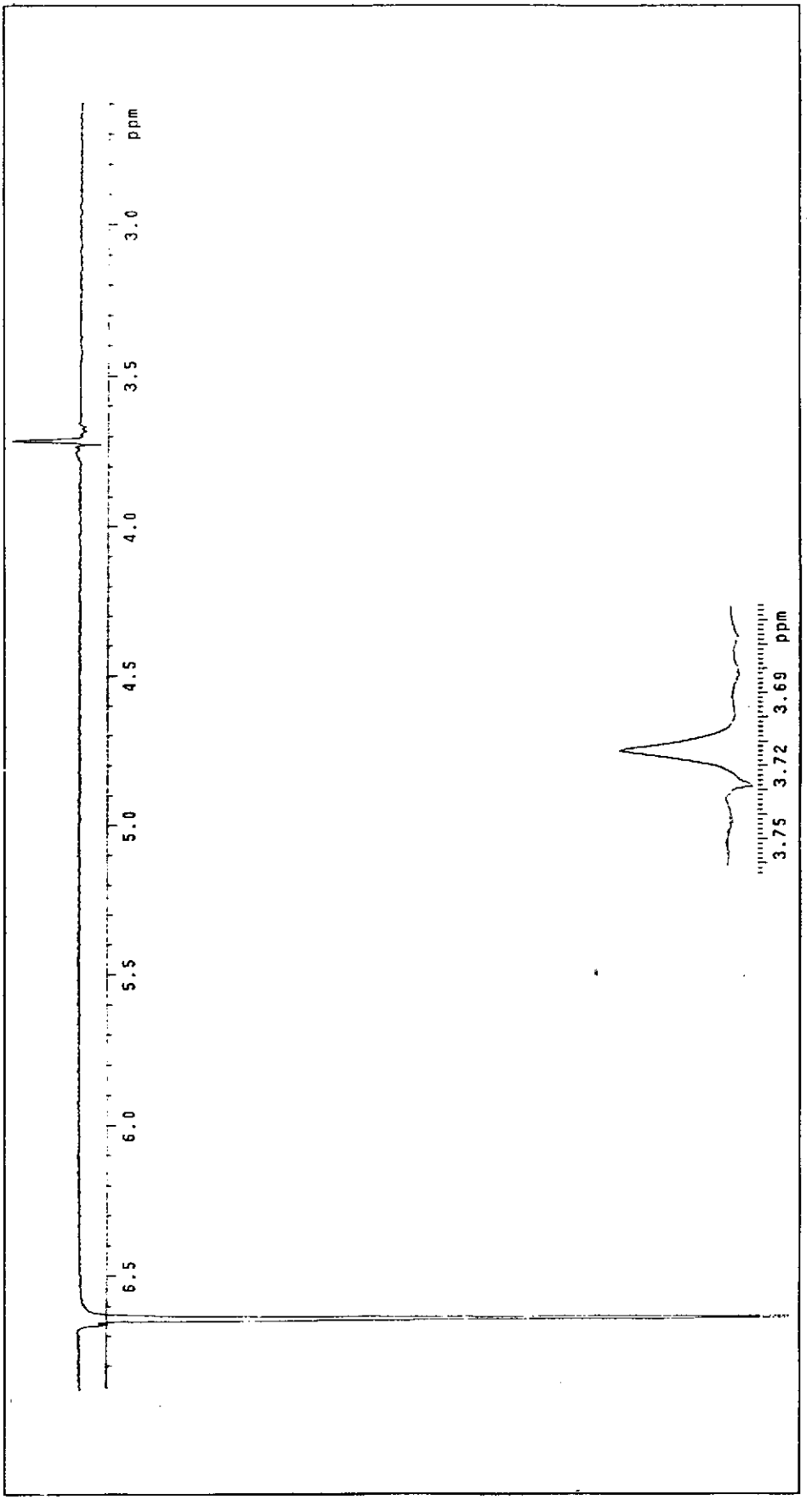


Figure 6 NOEDIFF spectrum of VR-JOY2 after irradiation at δ_H6.64

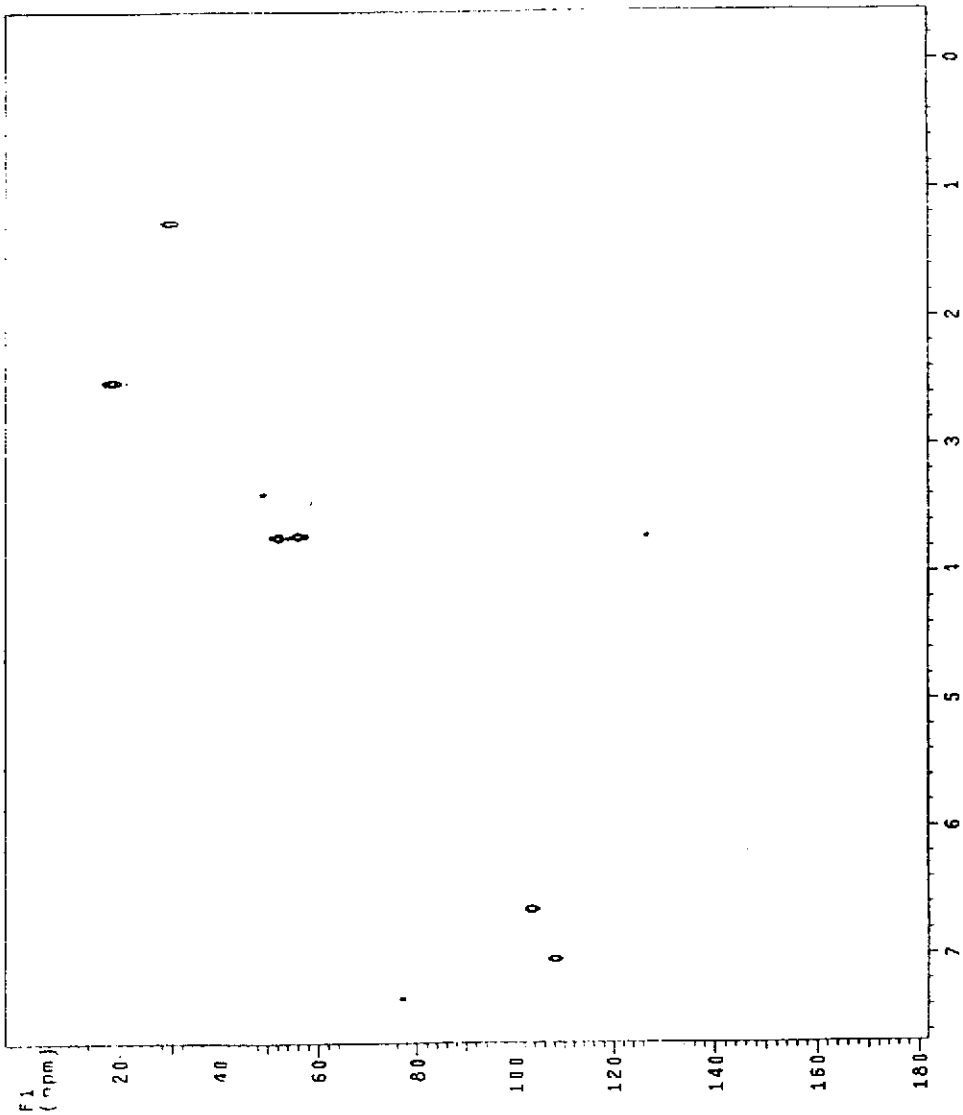


Figure 7 2D HMQC (500 MHz) spectrum of VR-JOY2

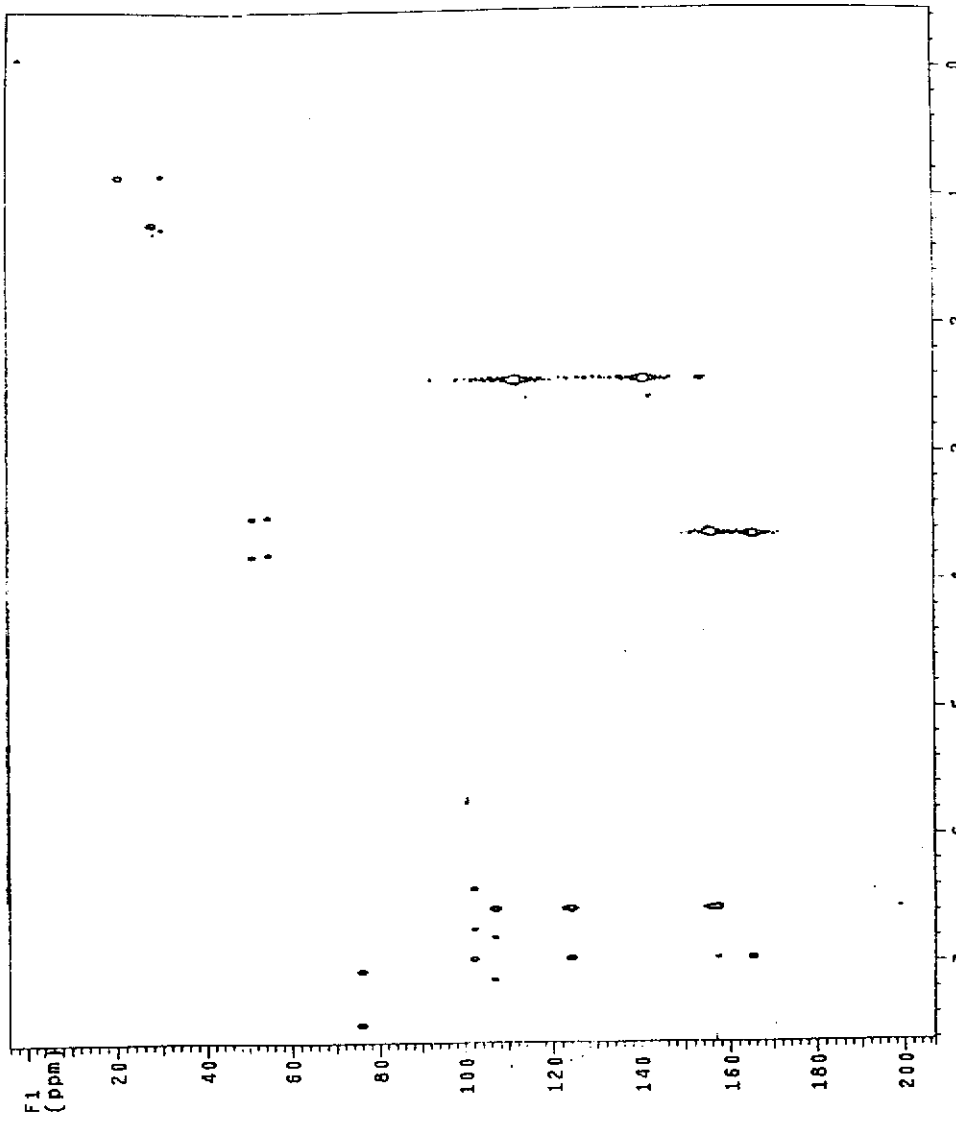


Figure 8 2D HMBC (500 MHz) spectrum of VR-JOY2

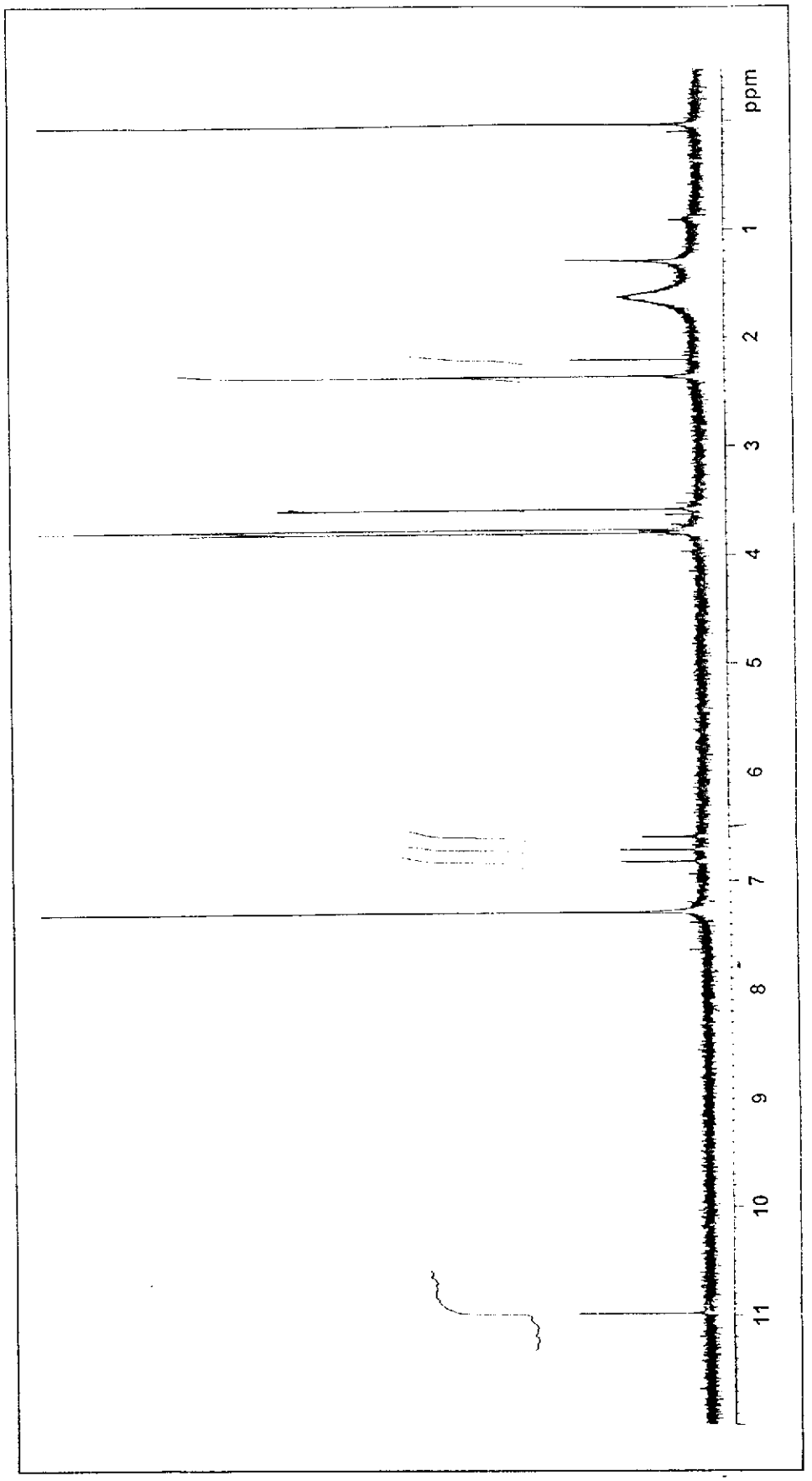


Figure 9 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of VR-JOY1

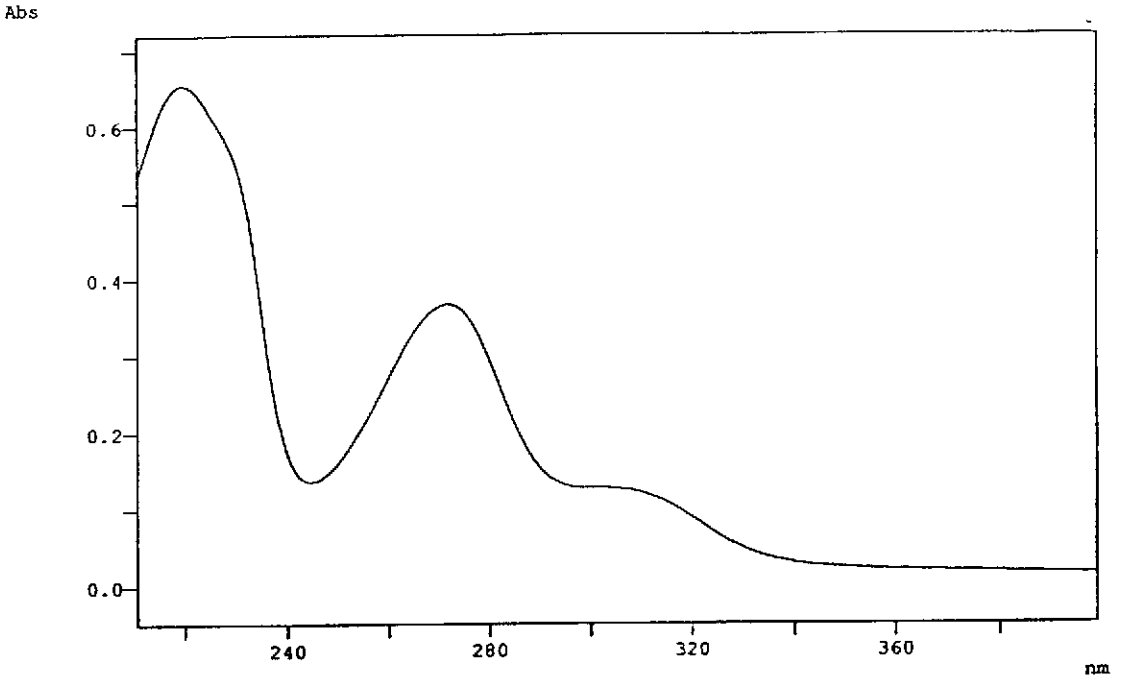


Figure 10 UV (MeOH) spectrum of VR-JOY3

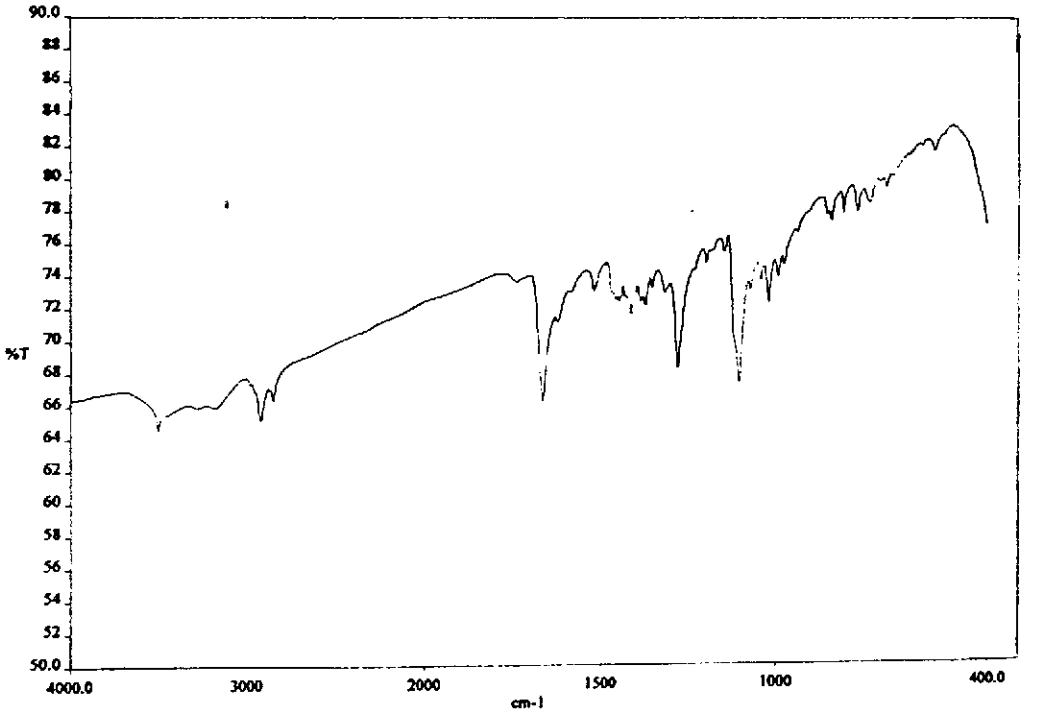


Figure 11 FT-IR (KBr) spectrum of VR-JOY3

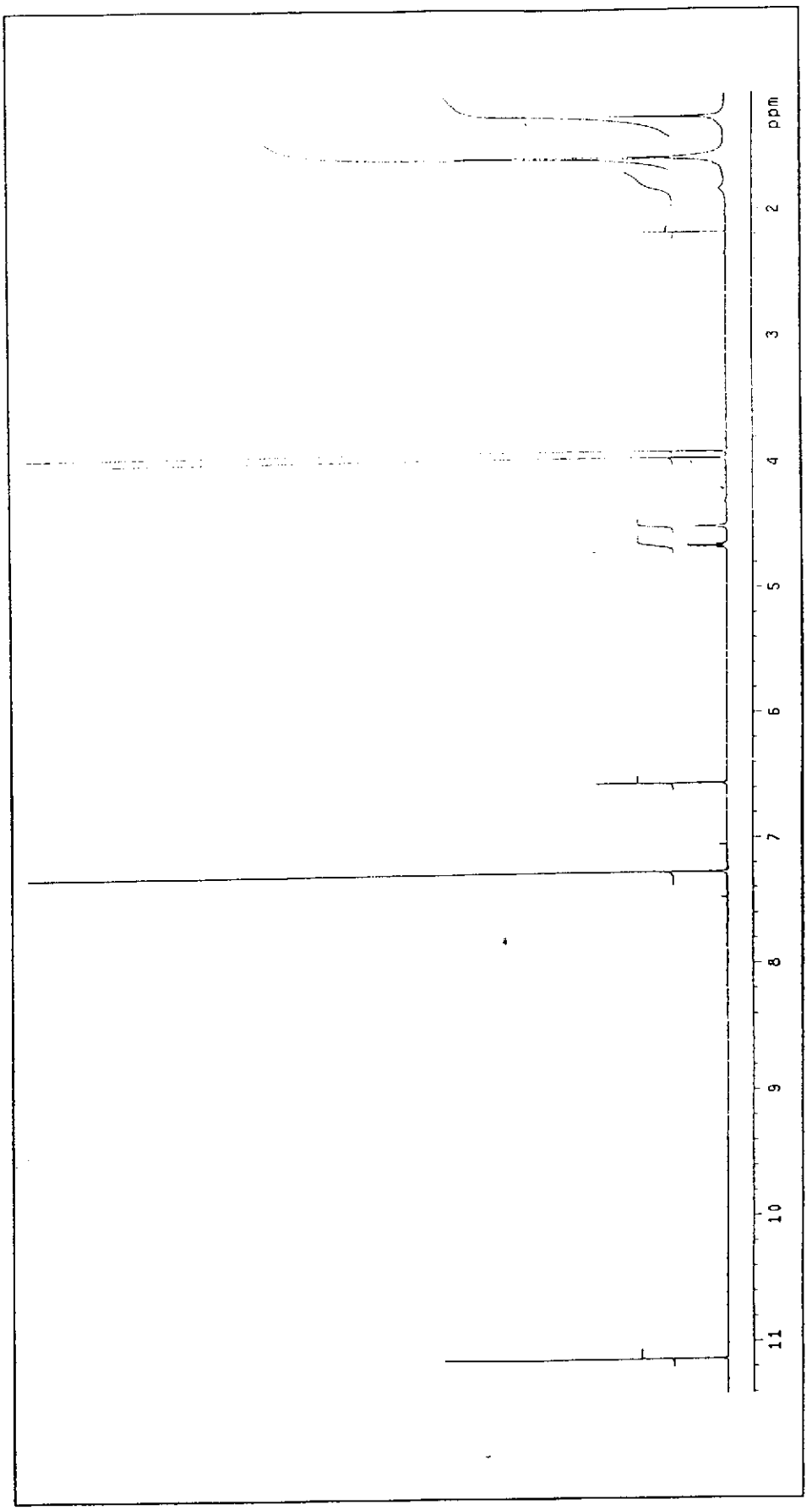


Figure 12 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of VR-JOY3

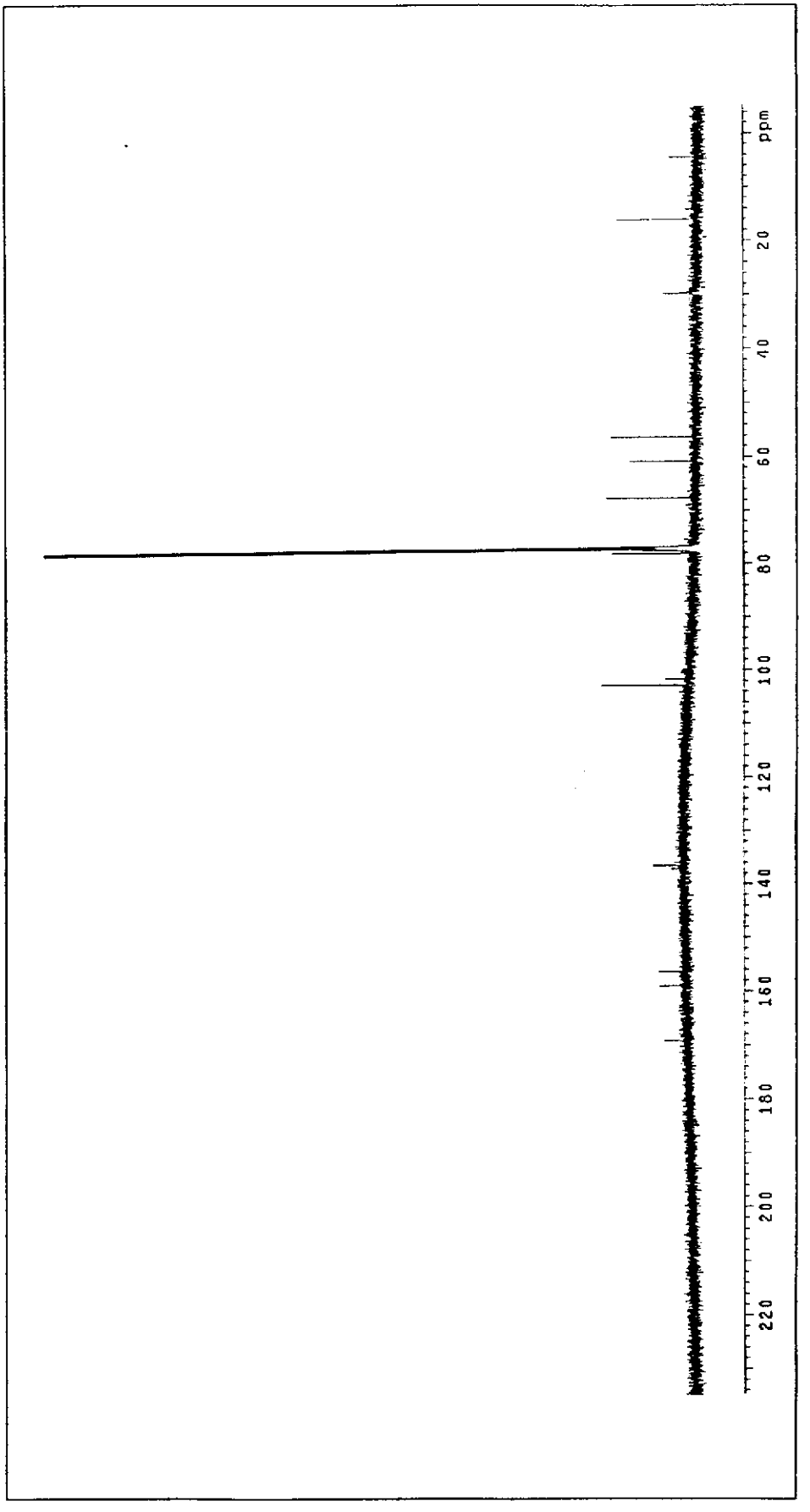


Figure 13 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of VR-JOY3

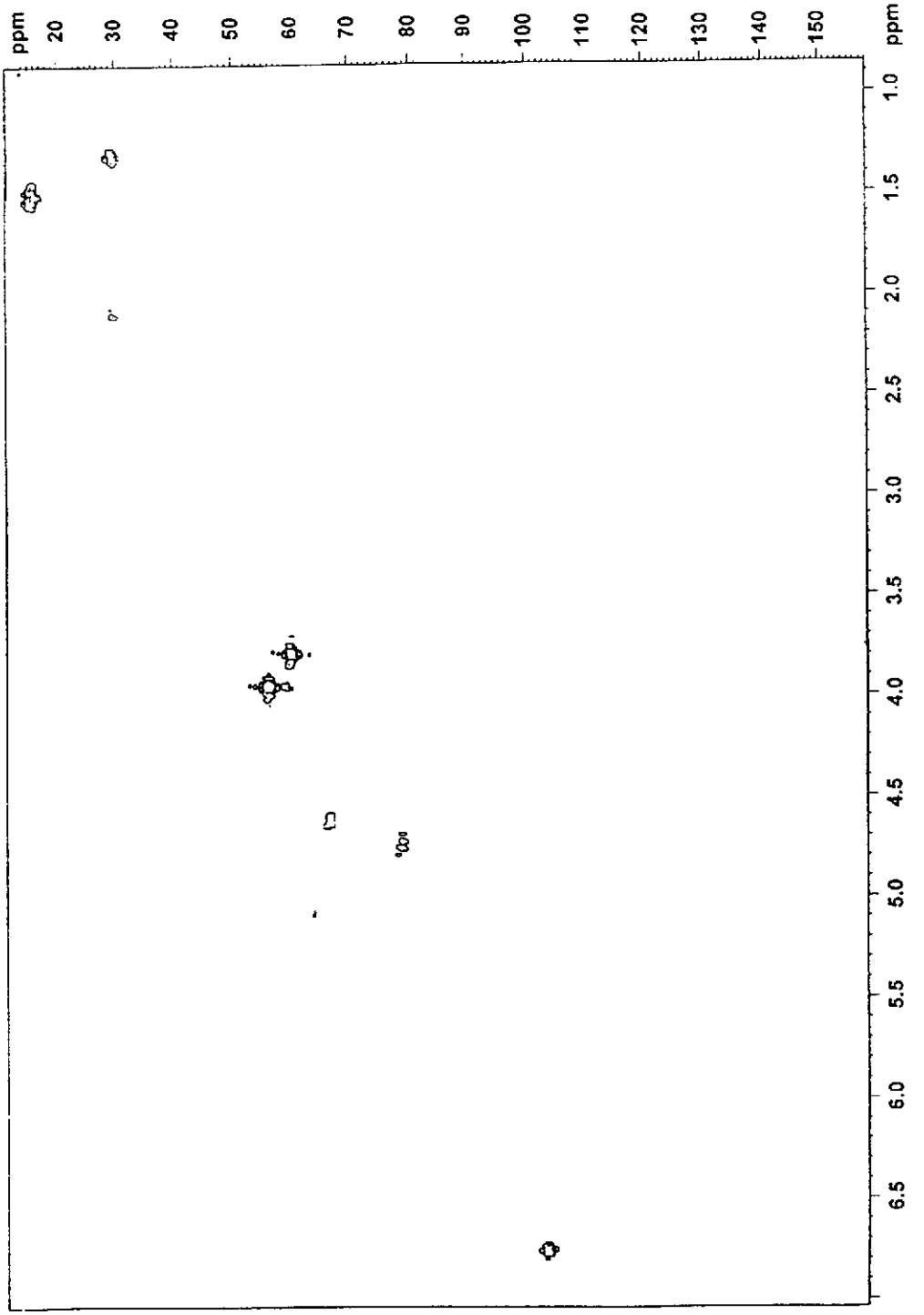


Figure 14 2D HMQC (300 MHz) spectrum of VR-JOY3

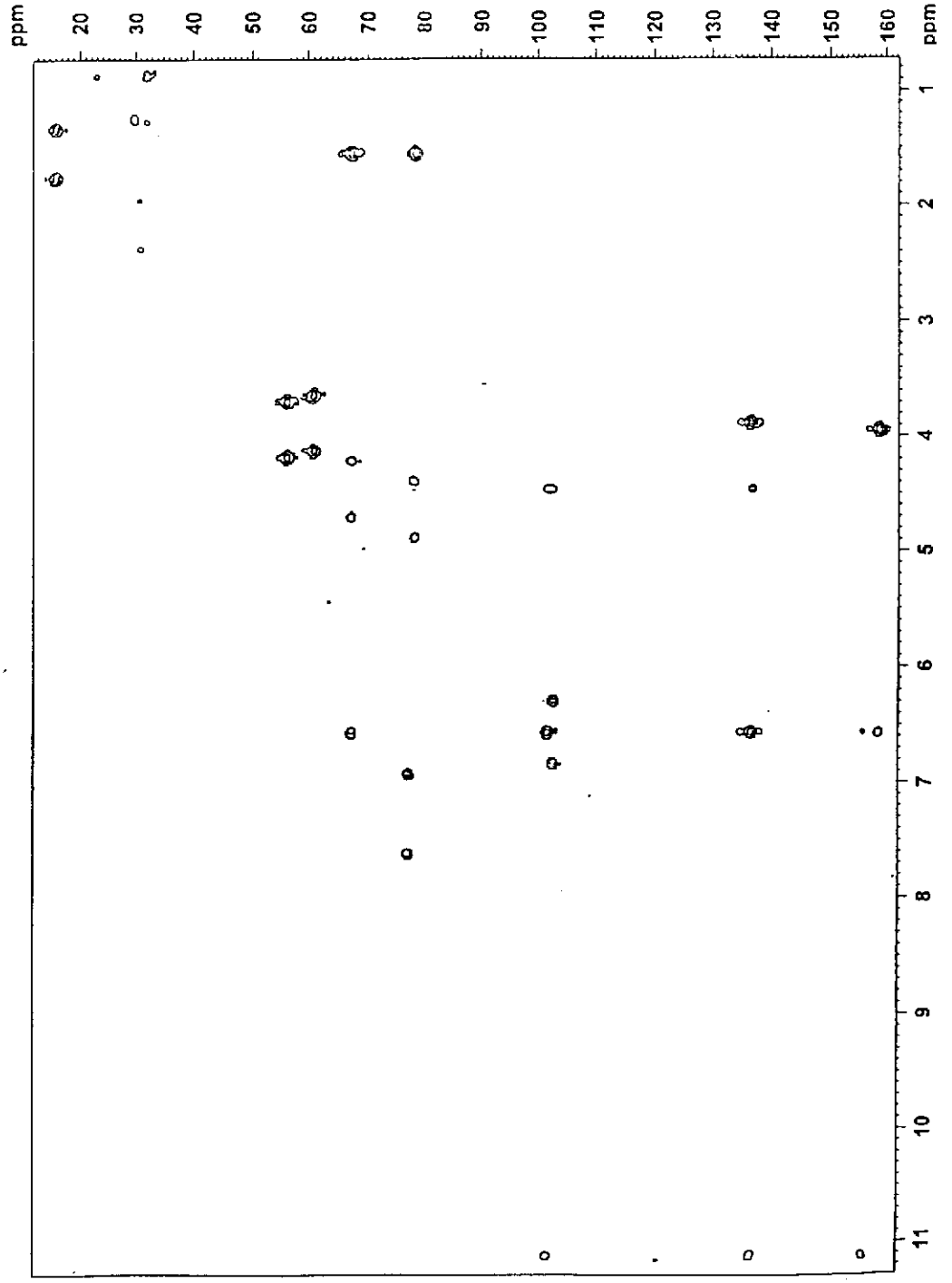


Figure 15 2D (300 MHz) HMBC spectrum of VR-JOY3

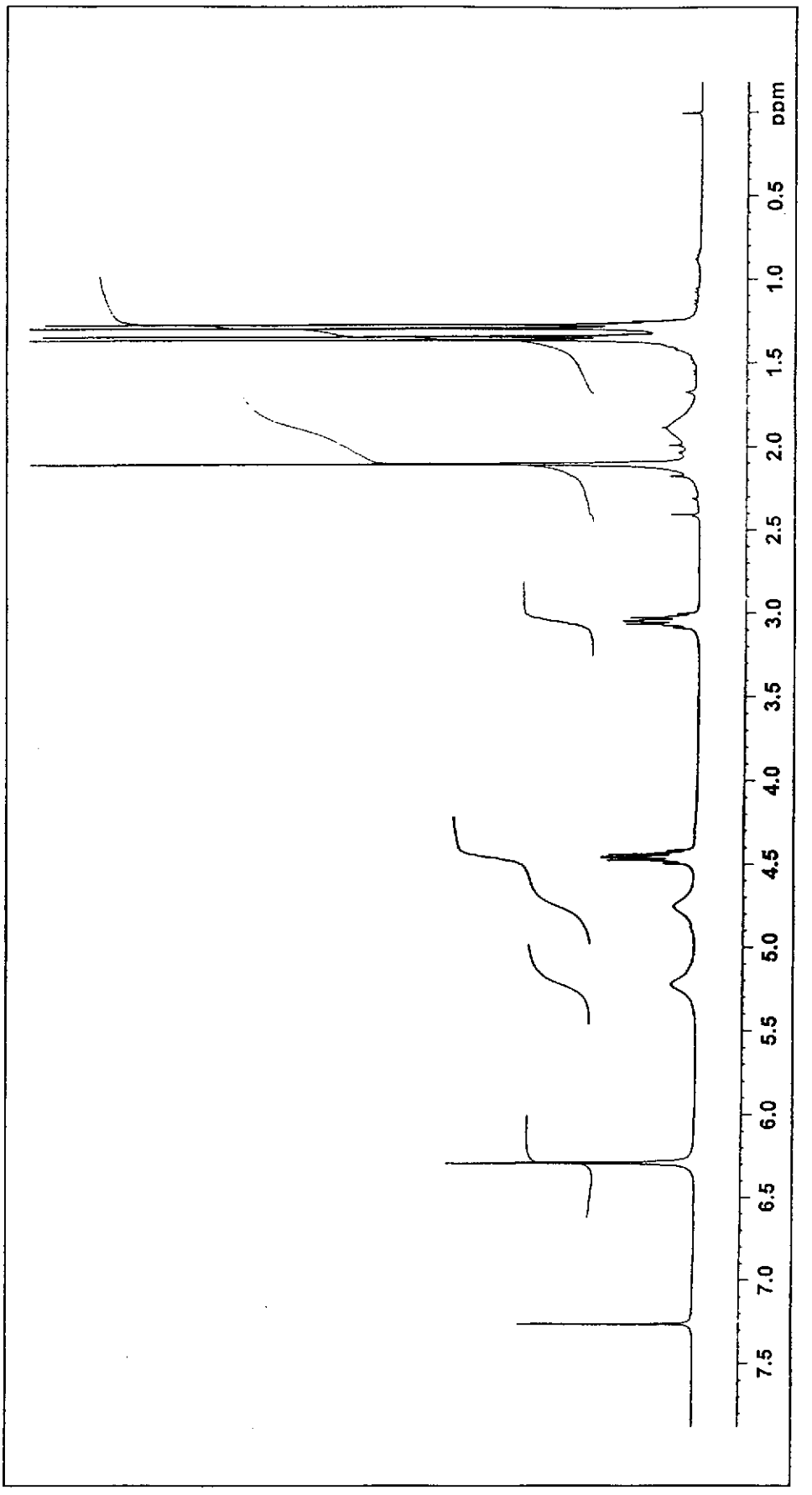


Figure 16 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of VR-JOY4

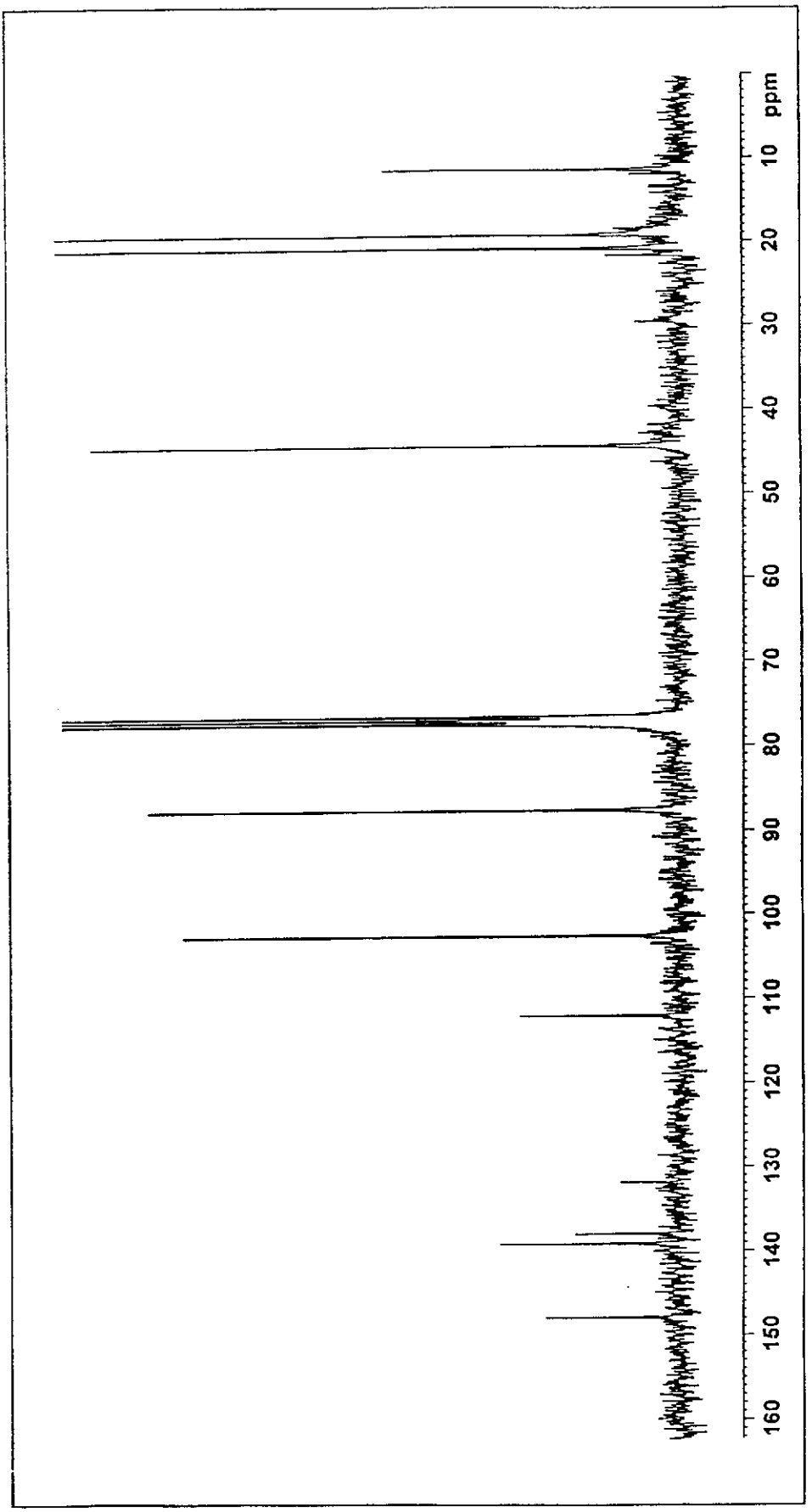


Figure 17 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of VR-JOY4

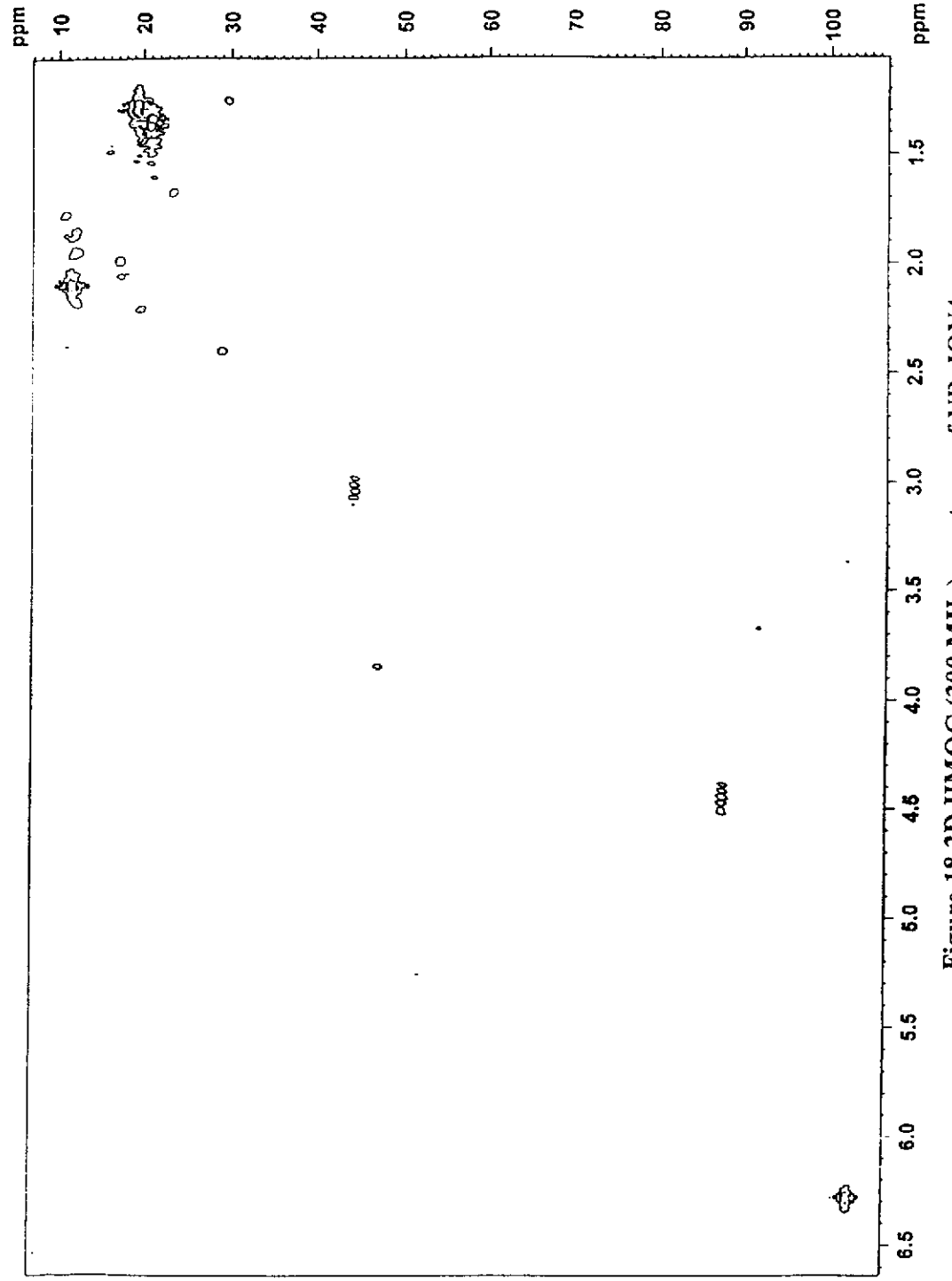


Figure 18 2D HMQC (300 MHz) spectrum of VR-JOY4

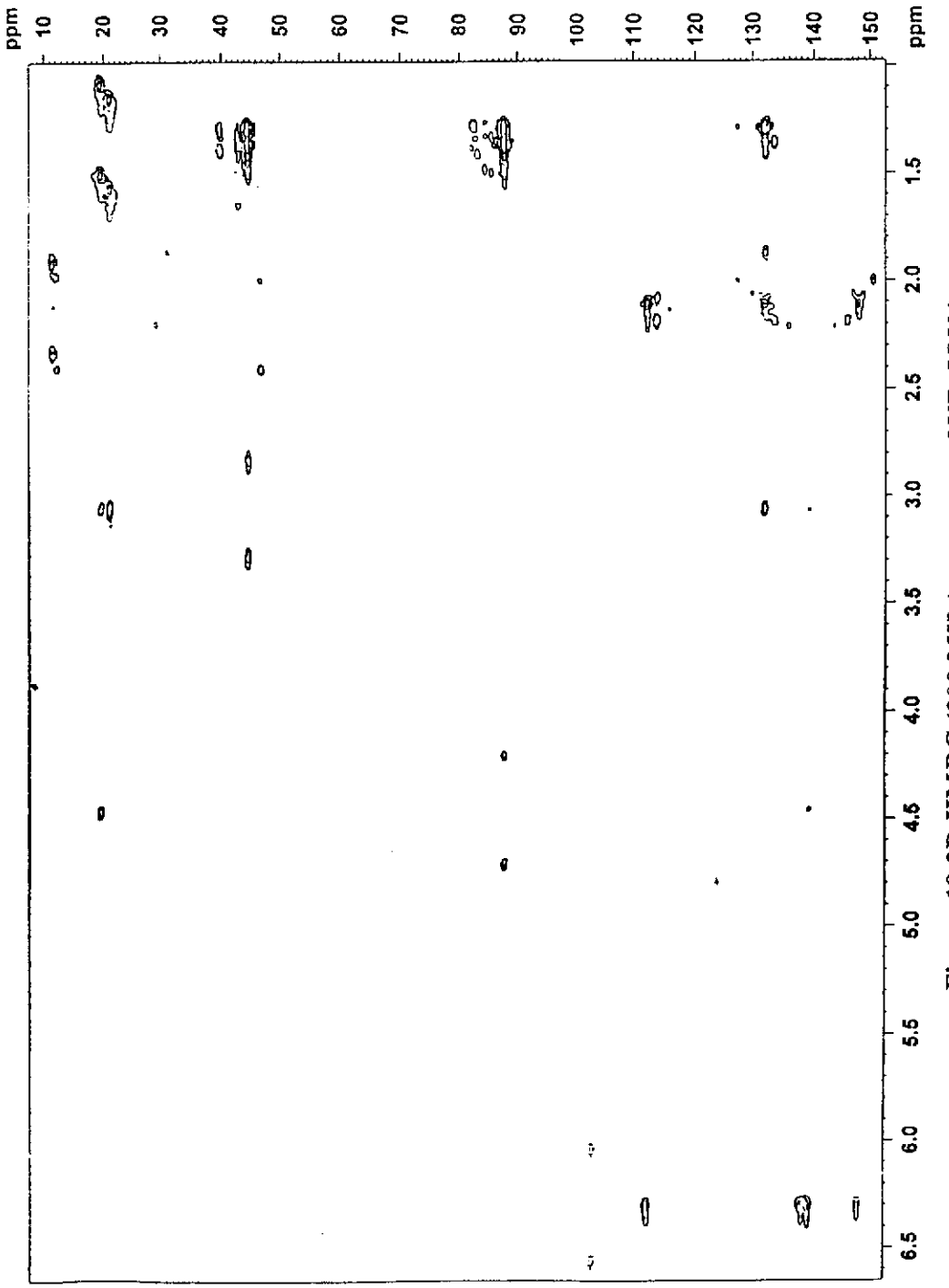


Figure 19 2D HMBC (300 MHz) spectrum of VR-JOY4

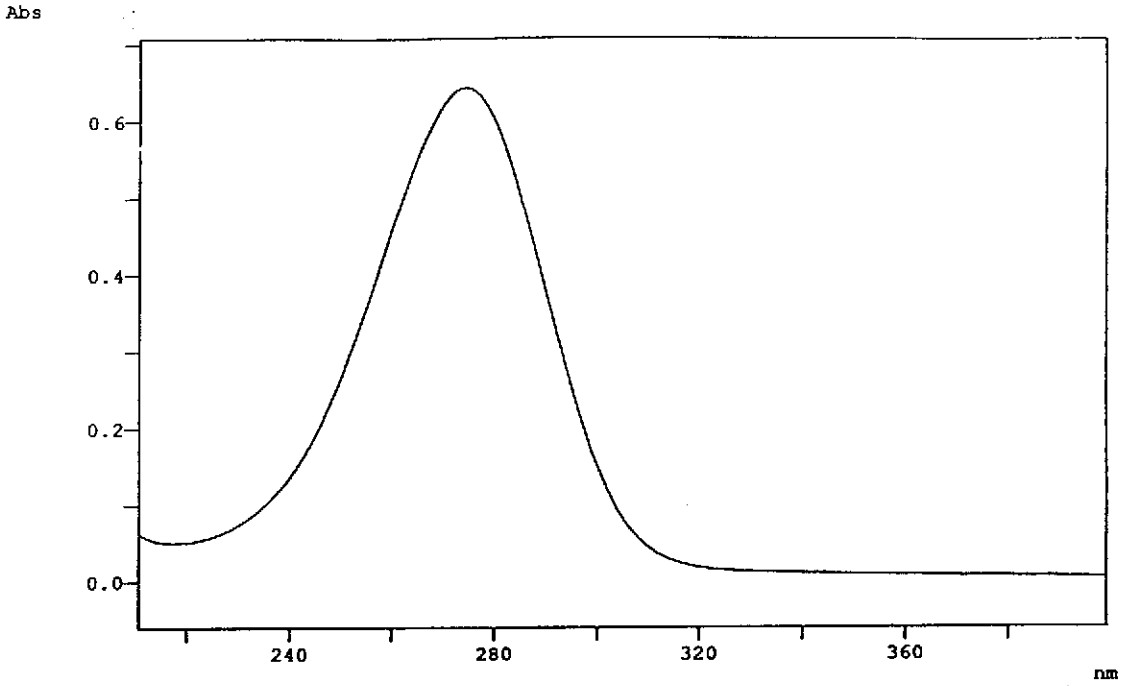


Figure 20 UV (MeOH) spectrum of VR-JOY5

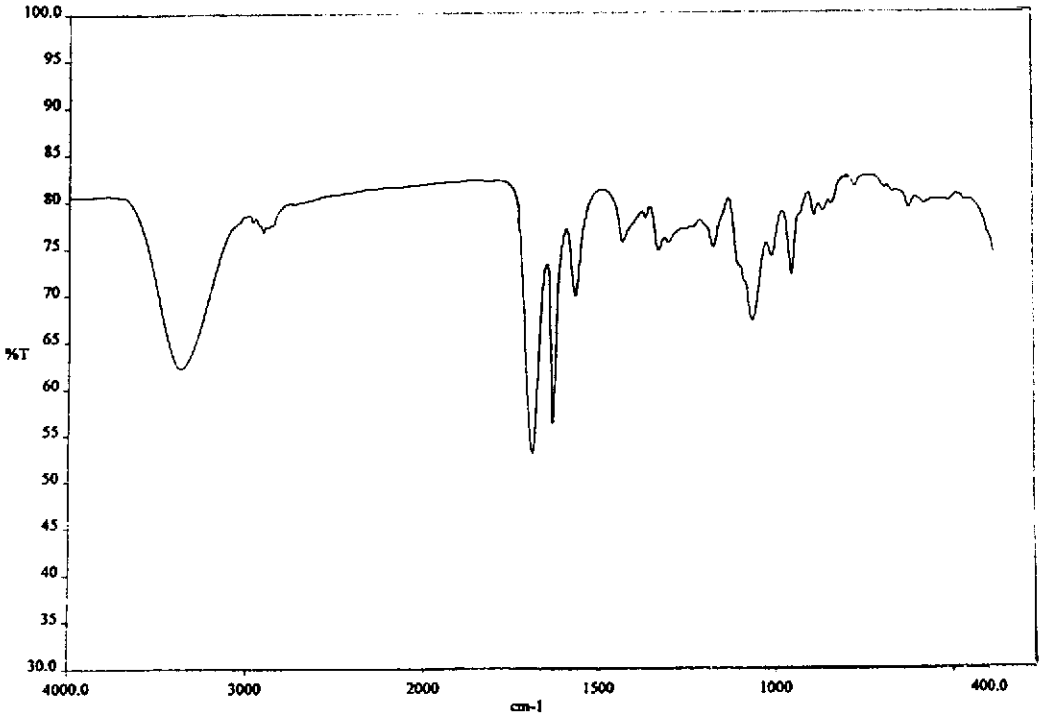


Figure 21 FT-IR (neat) spectrum of VR-JOY5

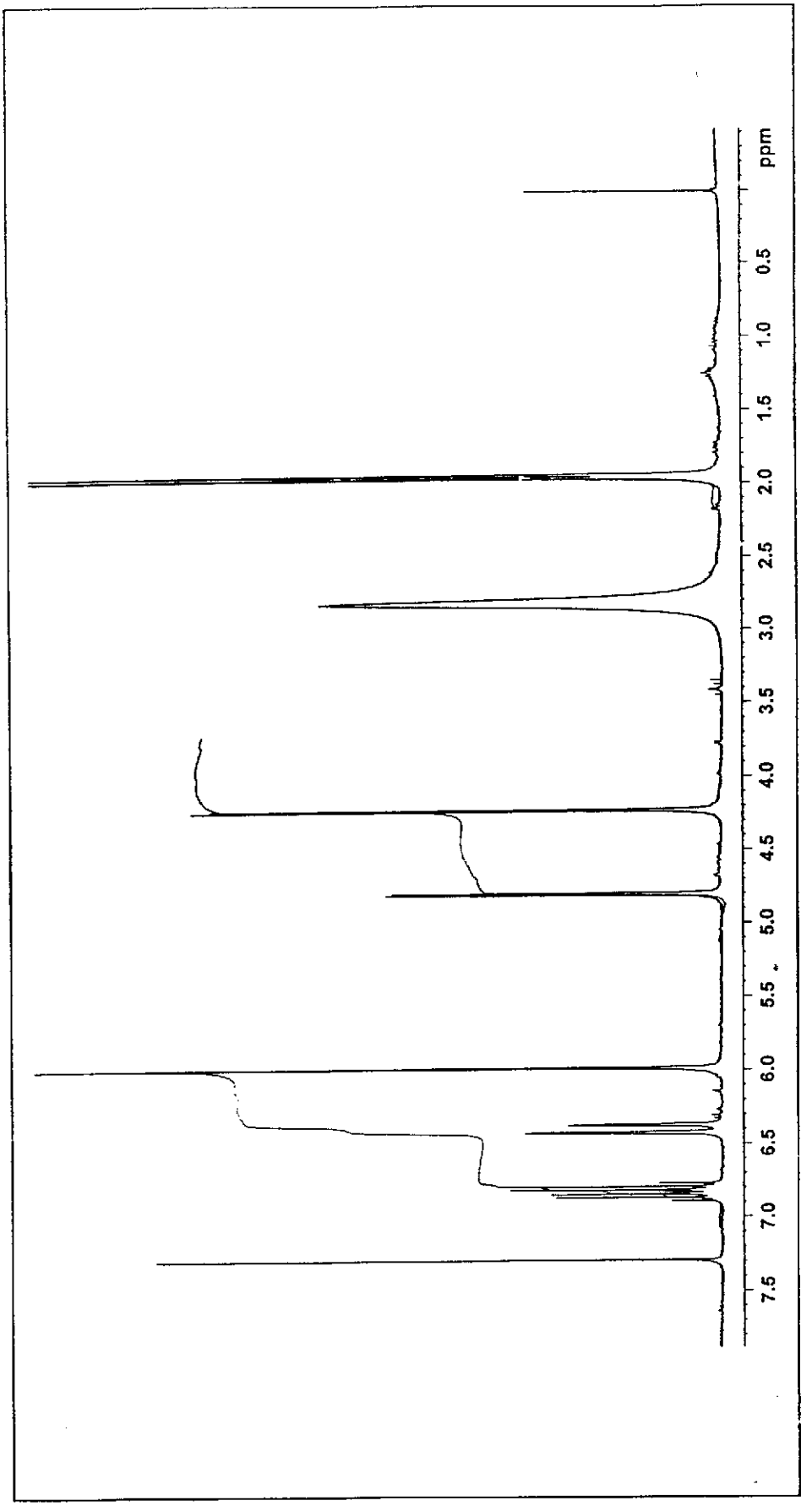


Figure 22 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of VR-JOY5

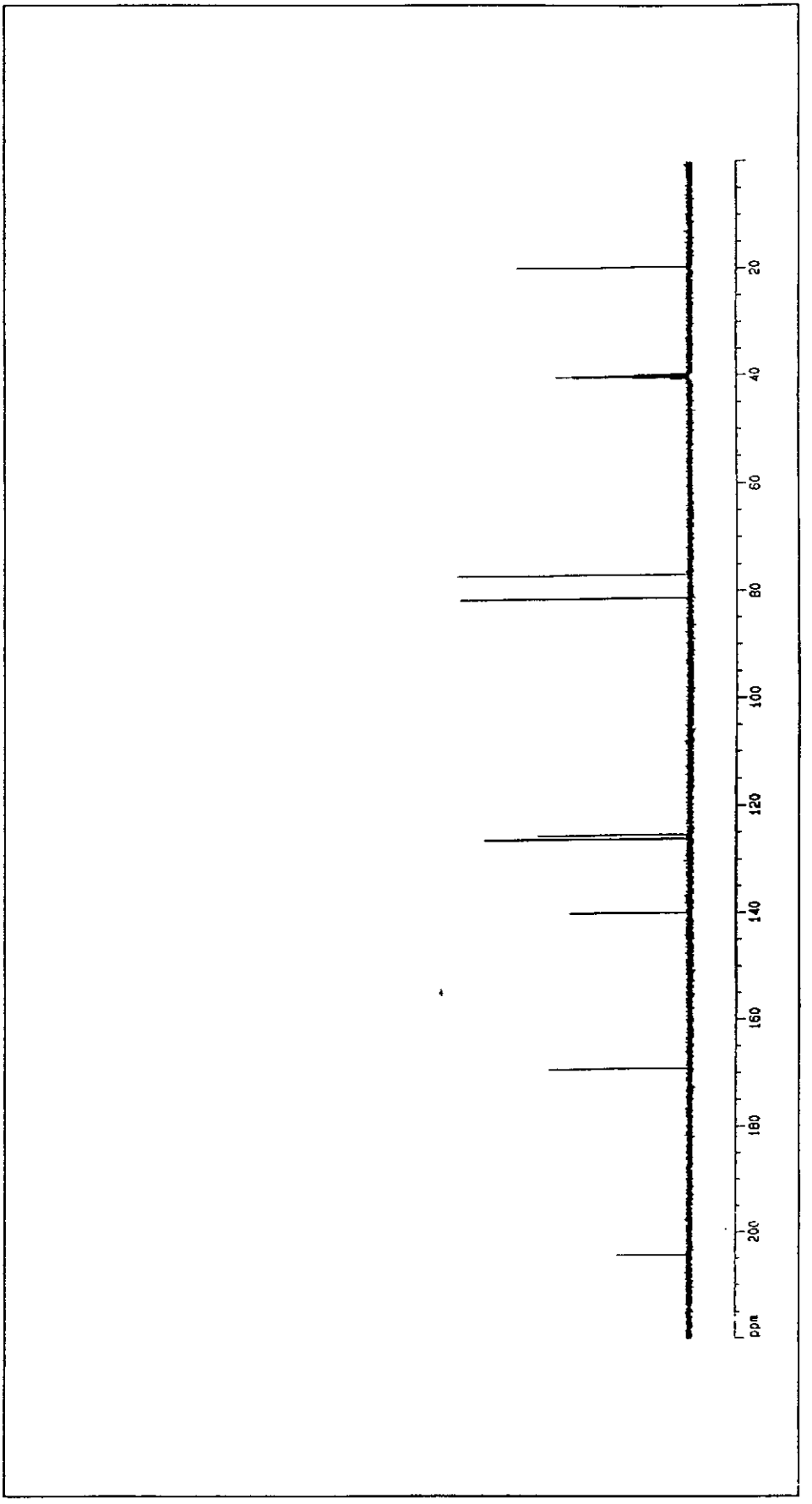


Figure 23 ^{13}C NMR (75 MHz) (Acetnone-d_6) spectrum of VR-JOY5

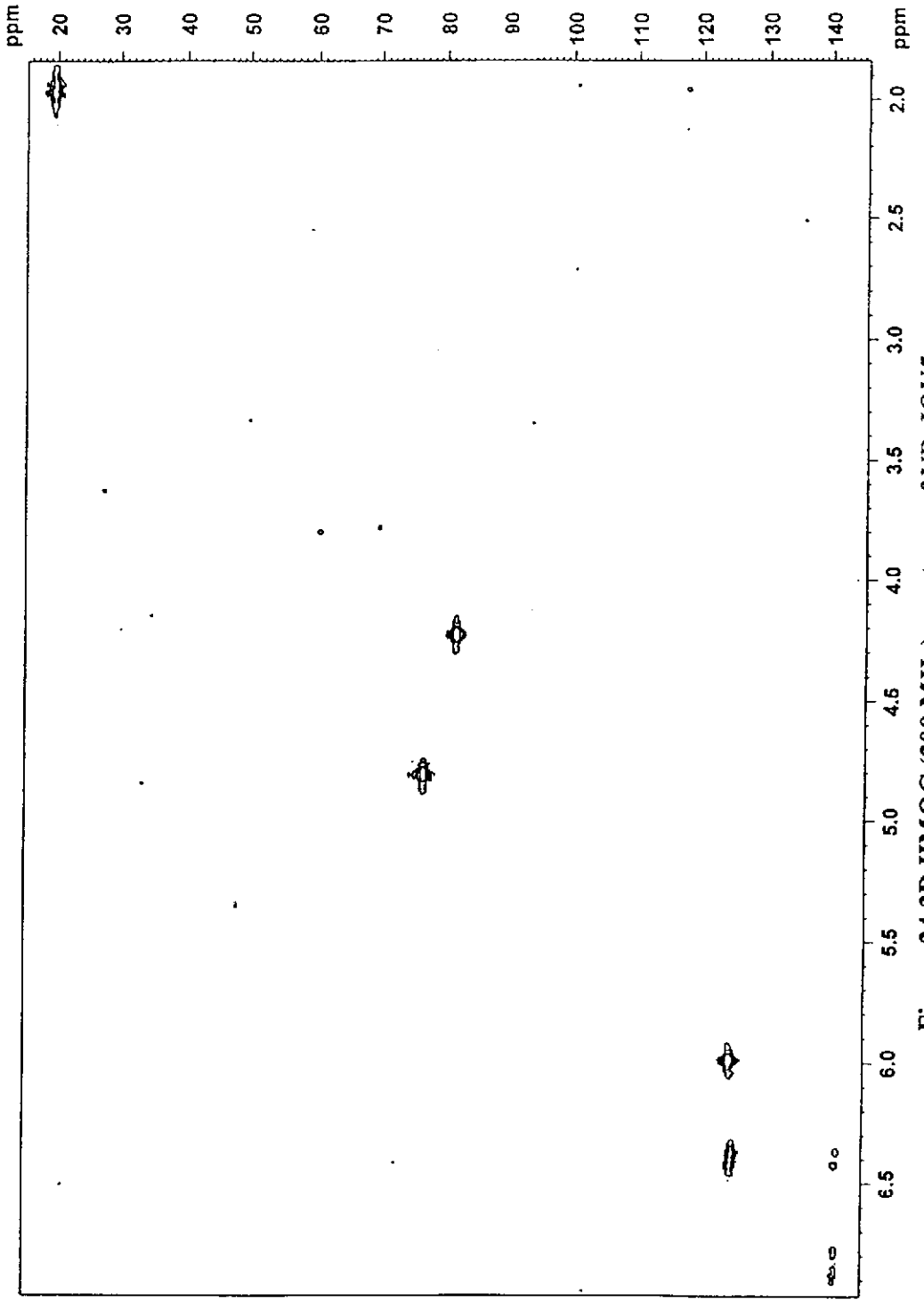


Figure 24 2D HMQC (300 MHz) spectrum of VR-JOY5

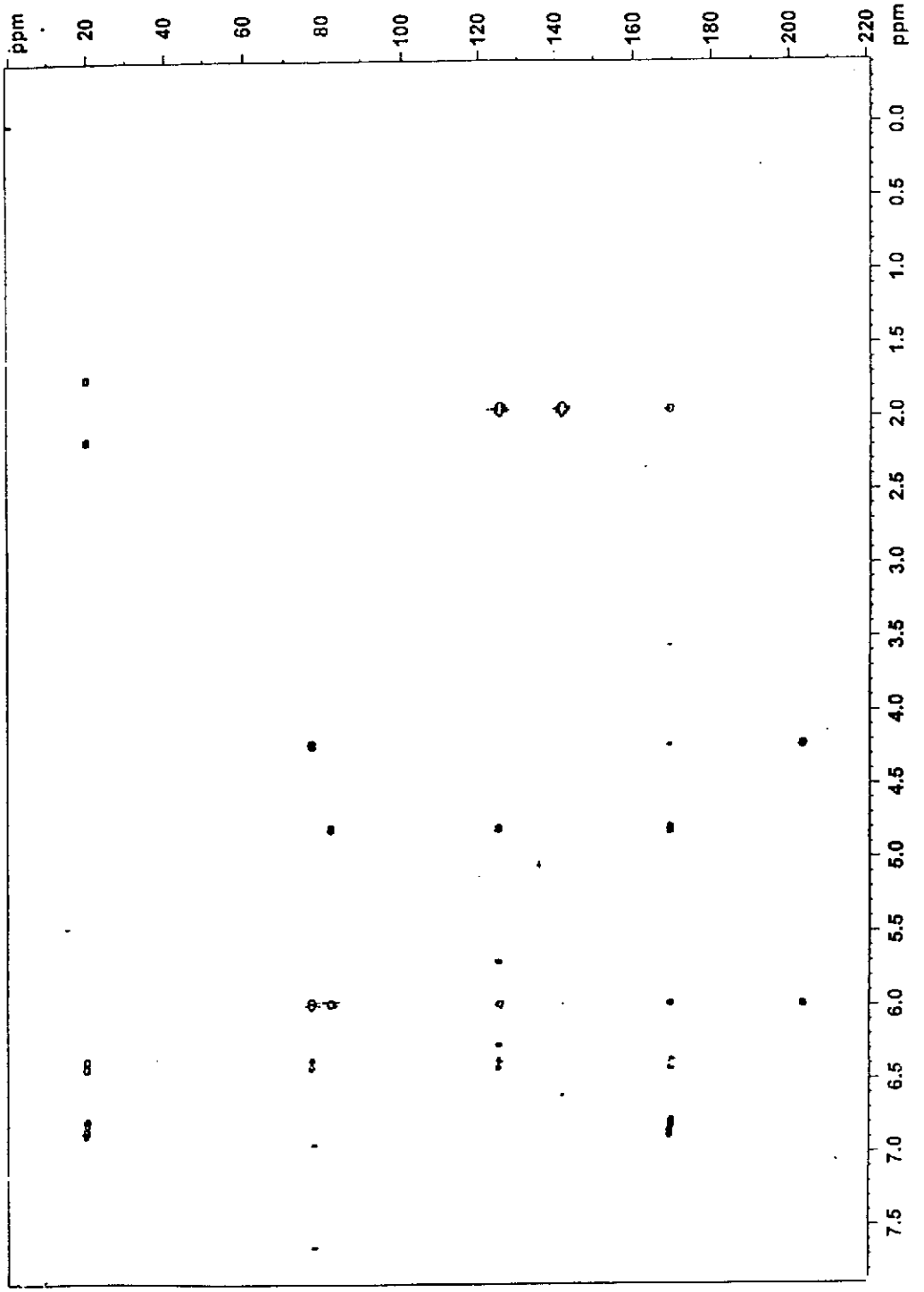


Figure 25 2D HMBC (300 MHz) spectrum of VR-JOY5

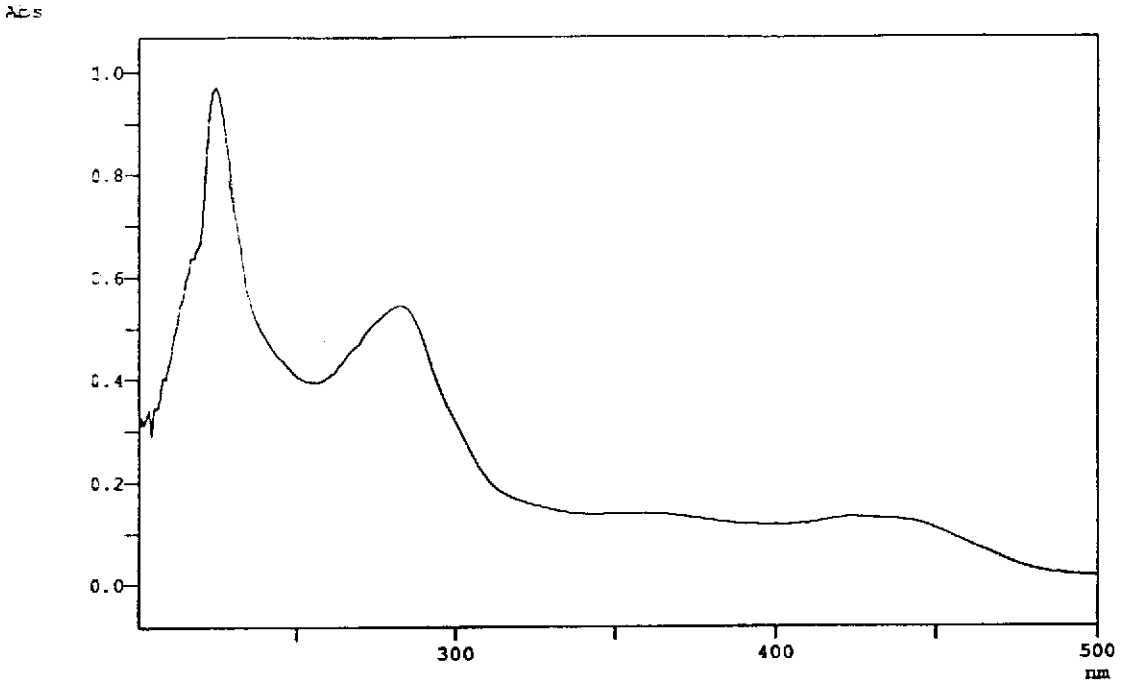


Figure 26 UV (MeOH) spectrum of VR-JOY6

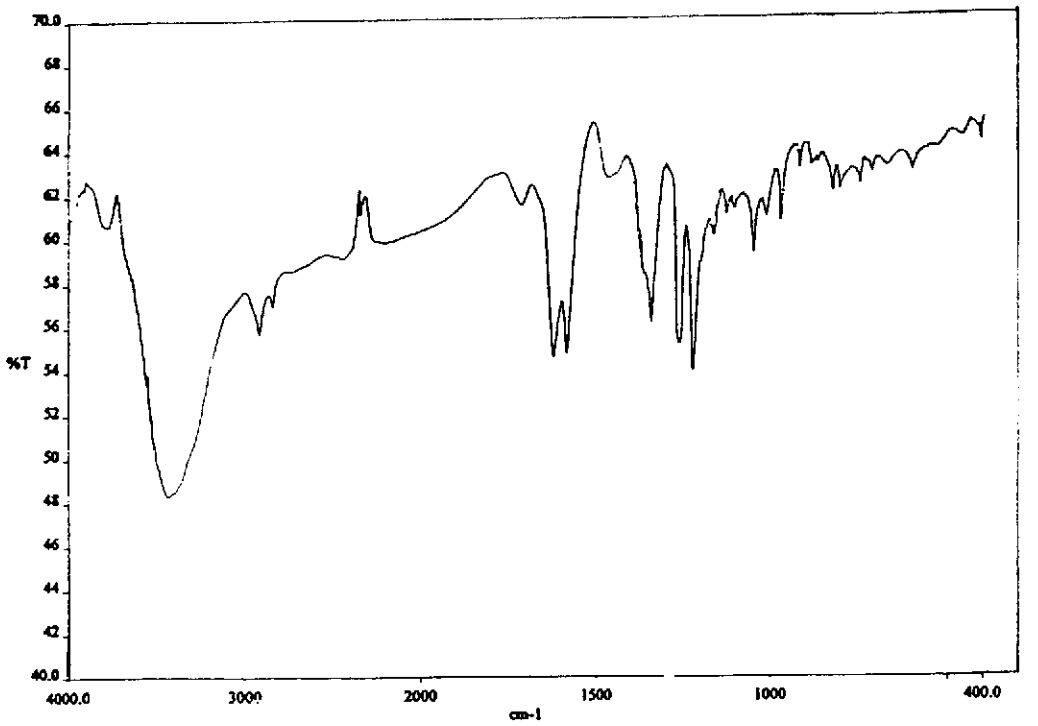


Figure 27 FT-IR (KBr) spectrum of VR-JOY6

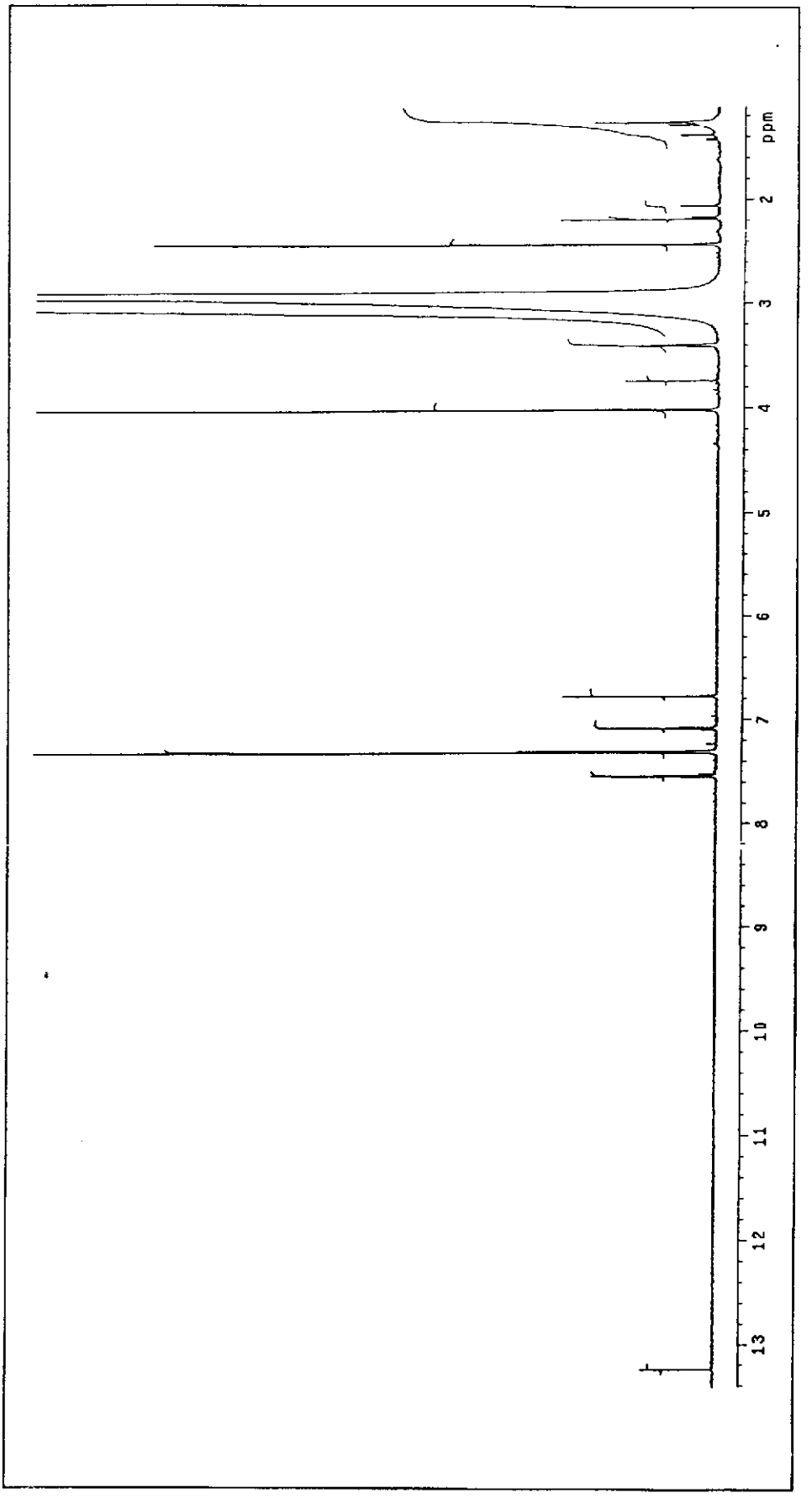


Figure 28 ^{1}H NMR (500 MHz) ($CDCl_3$+CD_3OD) spectrum of VR-JOY6

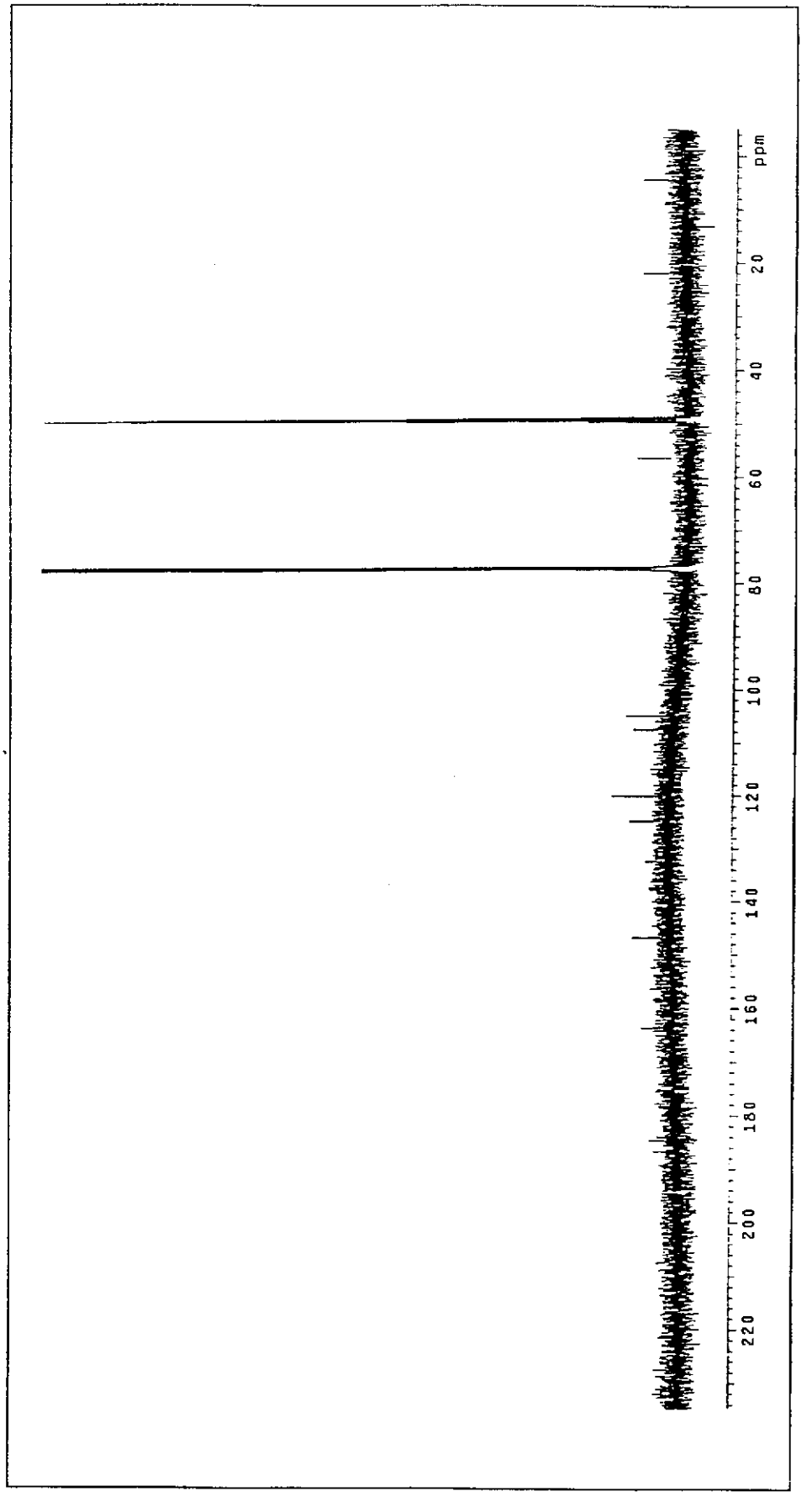


Figure 29 ^{13}C NMR (125 MHz) ($CDCl_3$+CD_3OD) spectrum of VR-JOY6

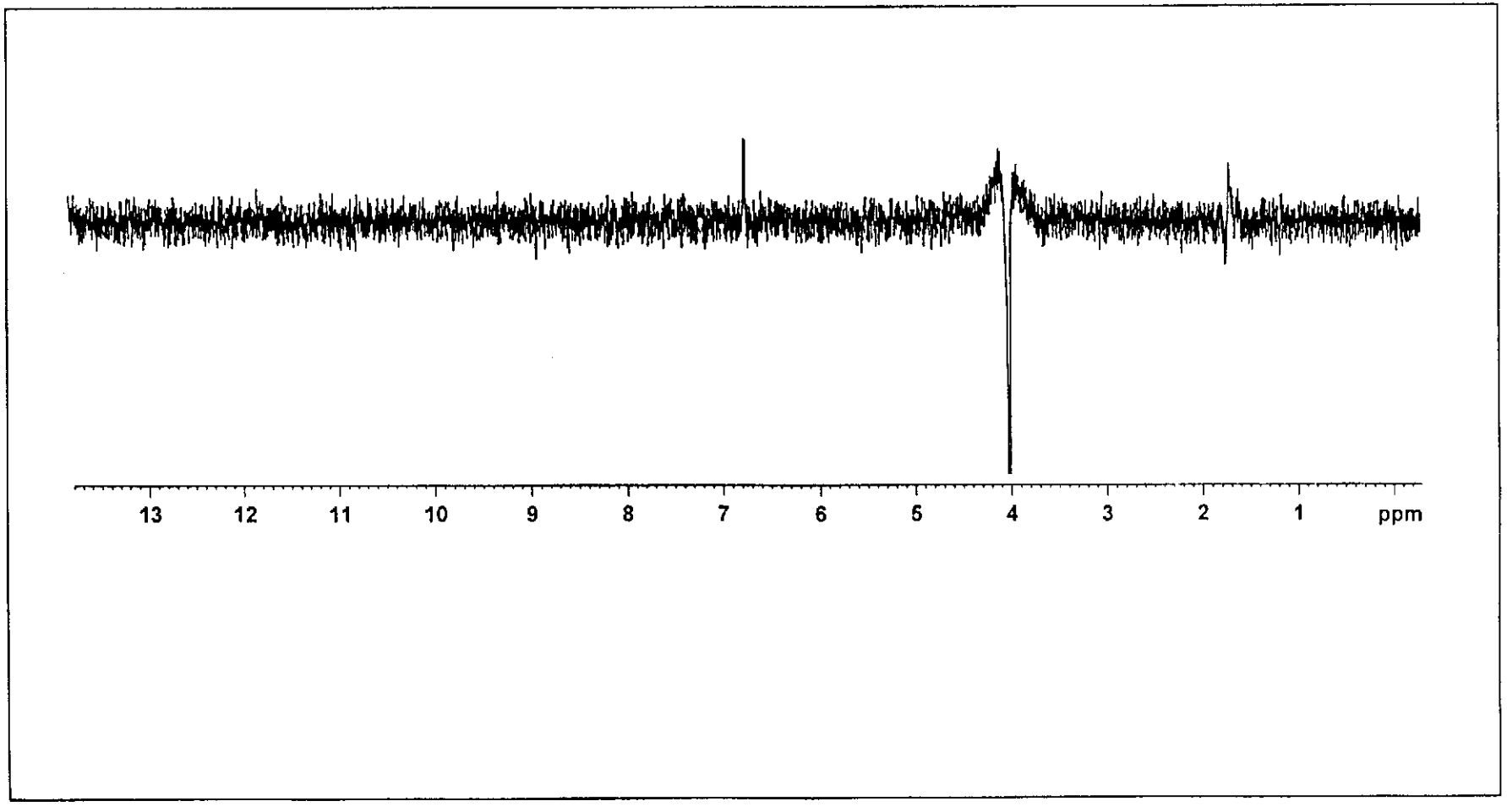


Figure 30 NOEDIFF spectrum of VR-JOY6 after irradiation at δ_H 4.01

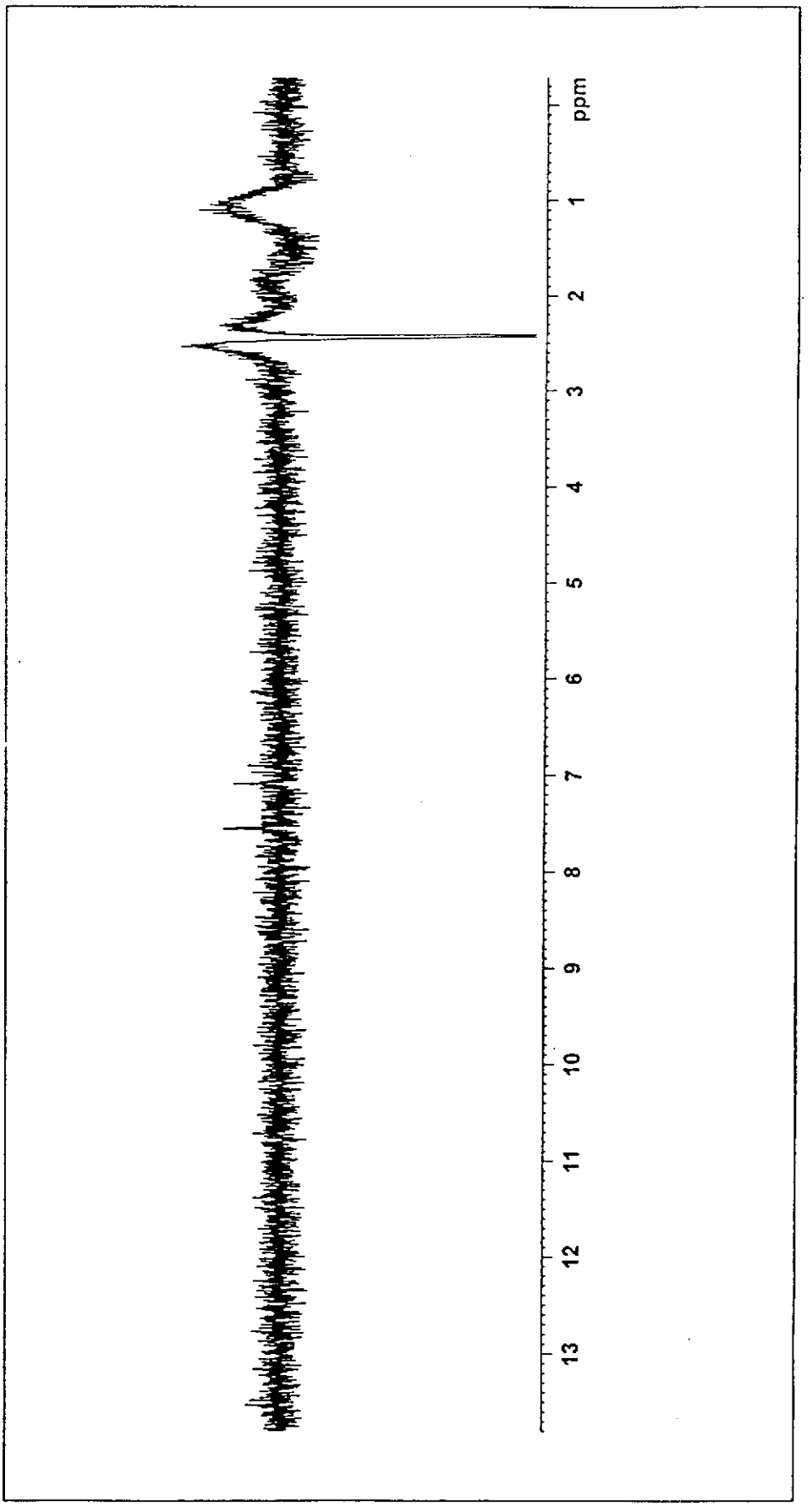


Figure 31 NOEDIFF spectrum of VR-JOY6 after irradiation at δ_H 2.43

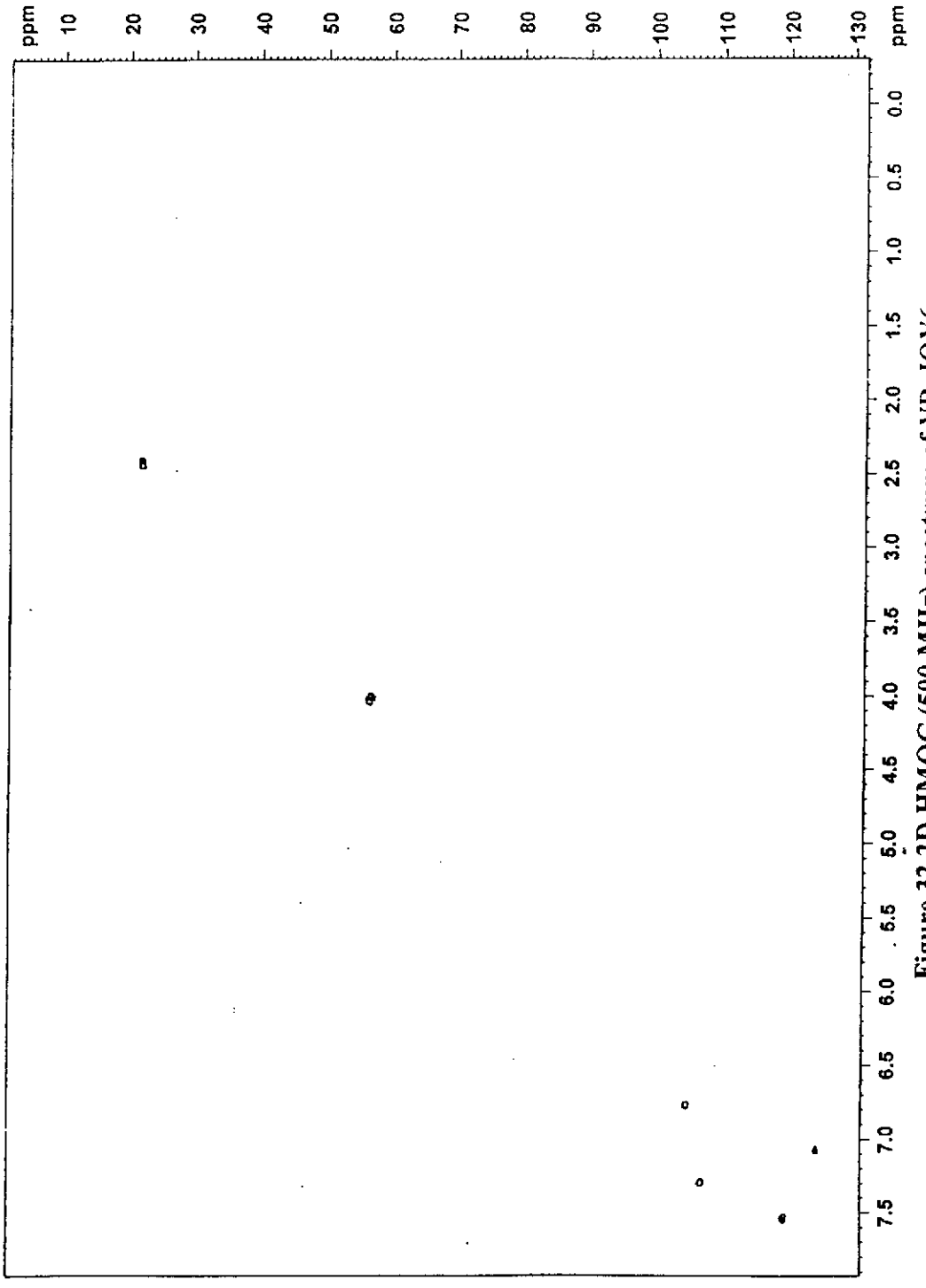


Figure 32 2D HMQC (500 MHz) spectrum of VR-JOY6

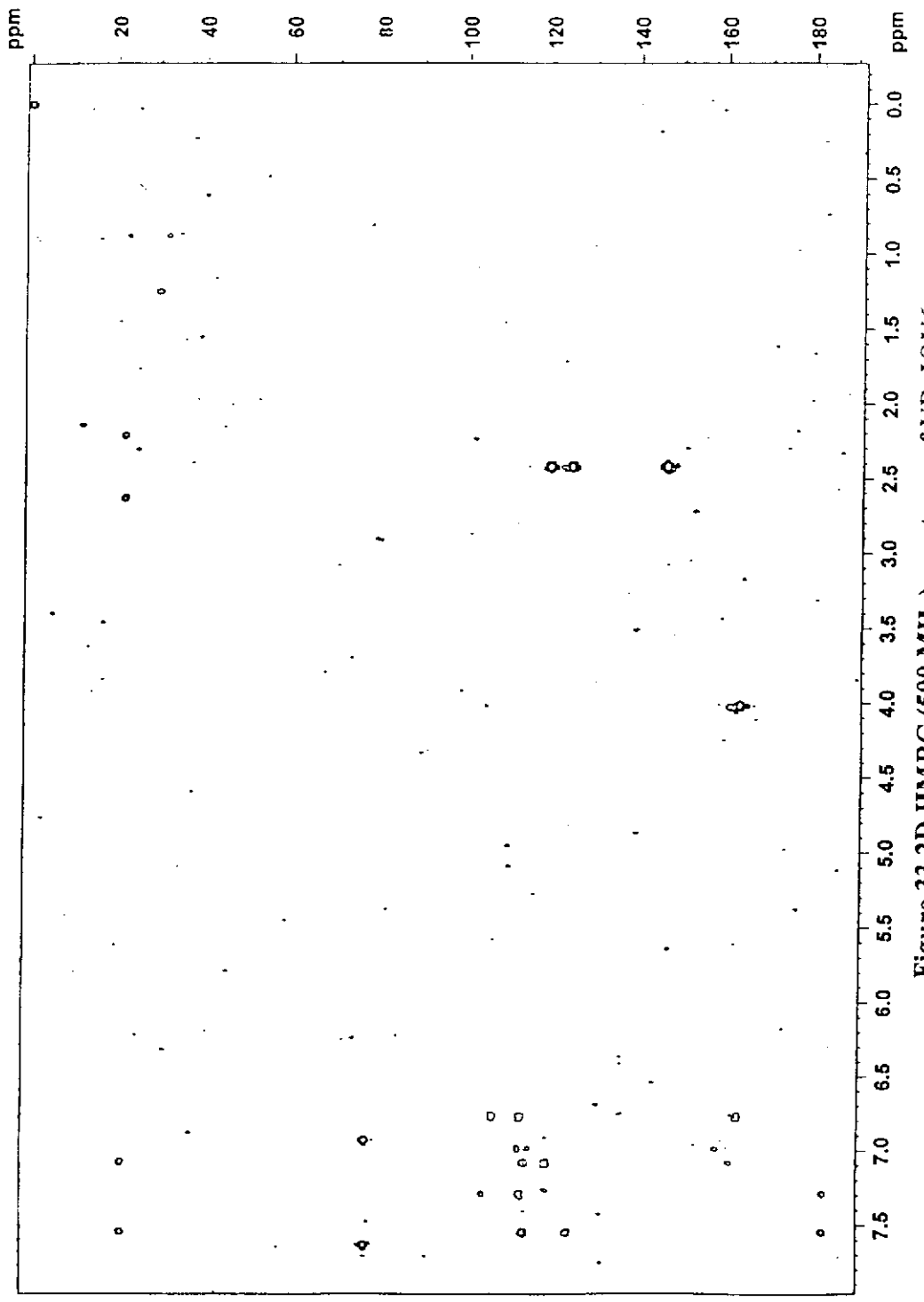


Figure 33 2D HMBC (500 MHz) spectrum of VR-JOY6

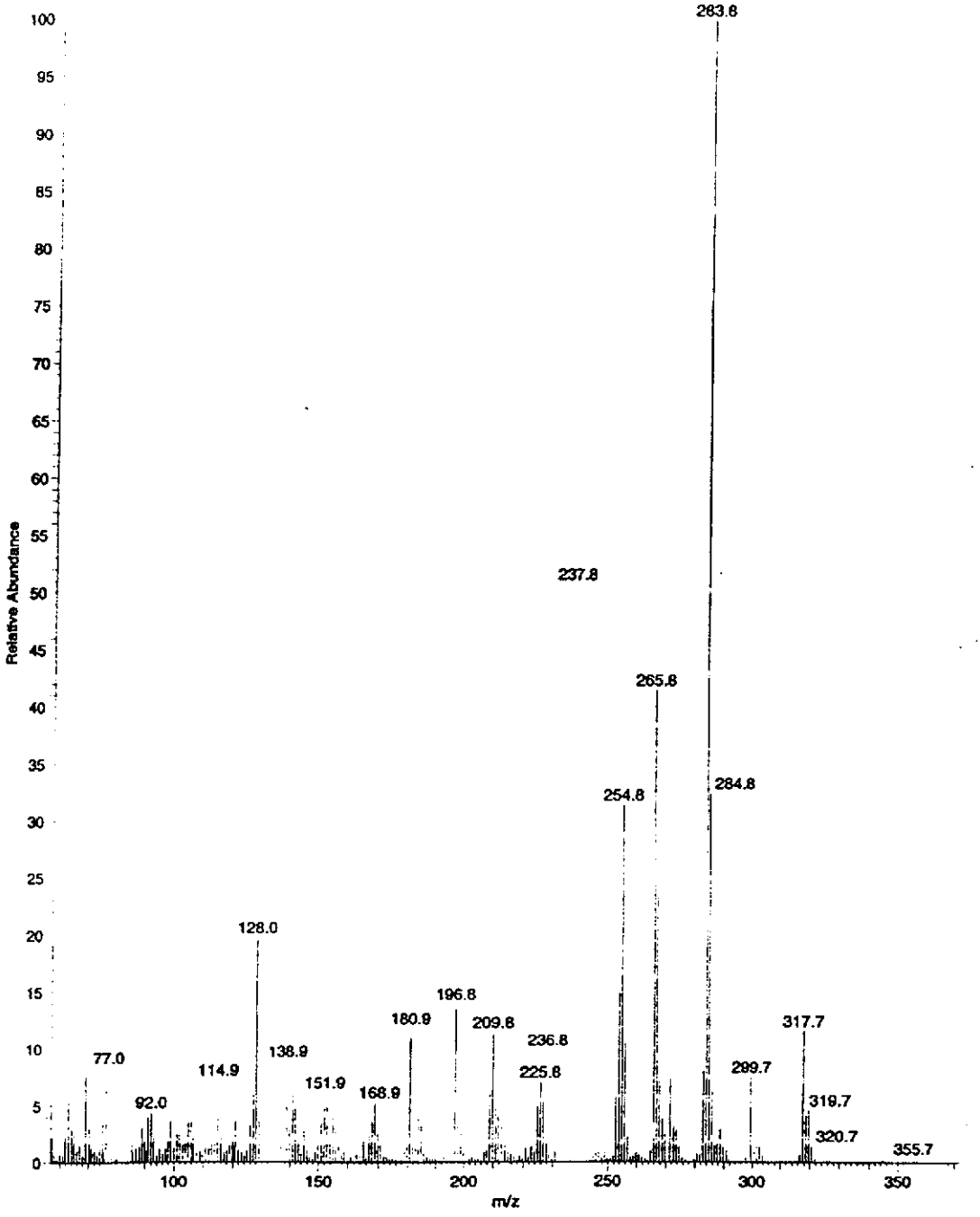


Figure 34 Mass spectrum of VR-JOY6

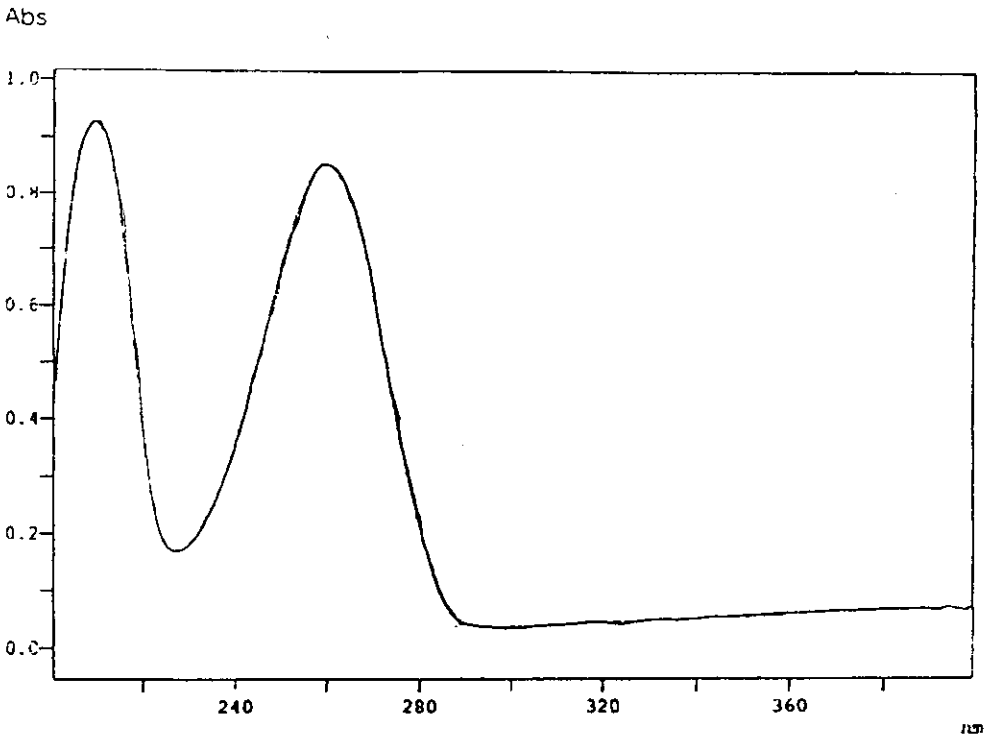


Figure 35 UV (MeOH) spectrum of VR-JOY10

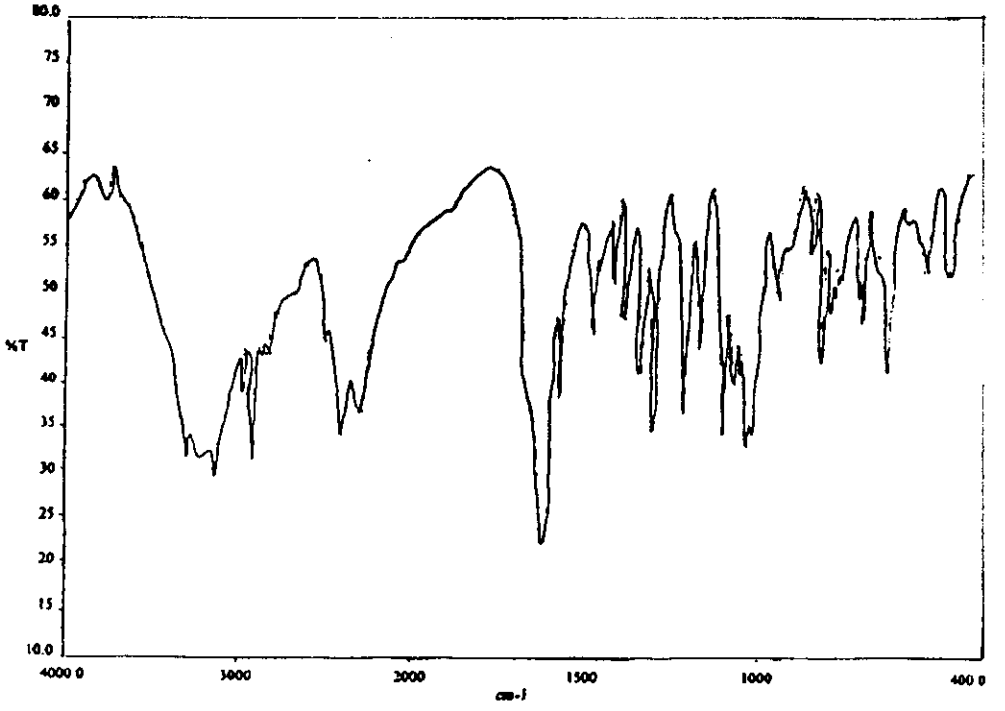


Figure 36 FT-IR (KBr) spectrum of VR-JOY10

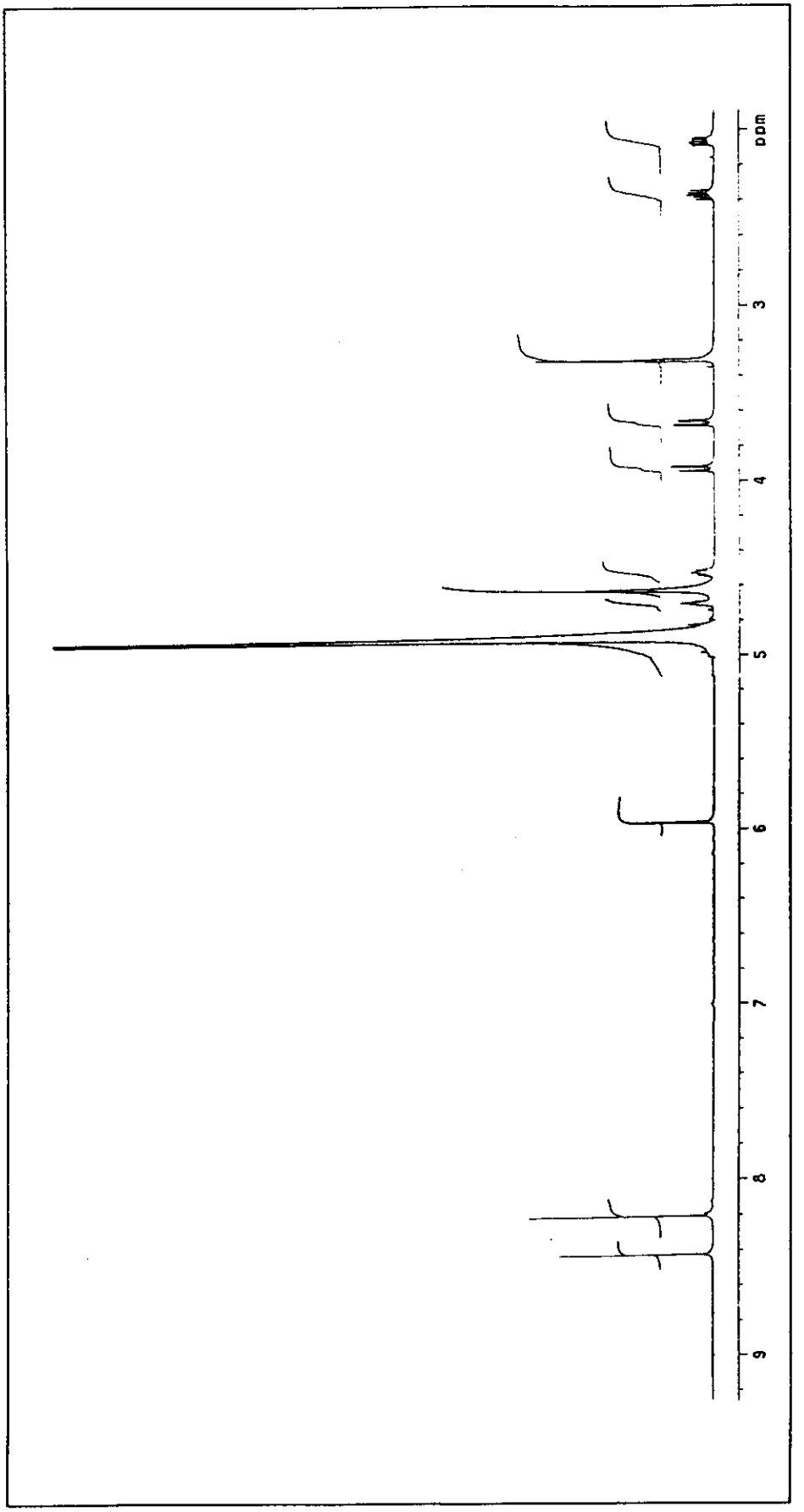


Figure 37 ^{1}H NMR (500 MHz) (CD_3OD) spectrum of VR-JOY10

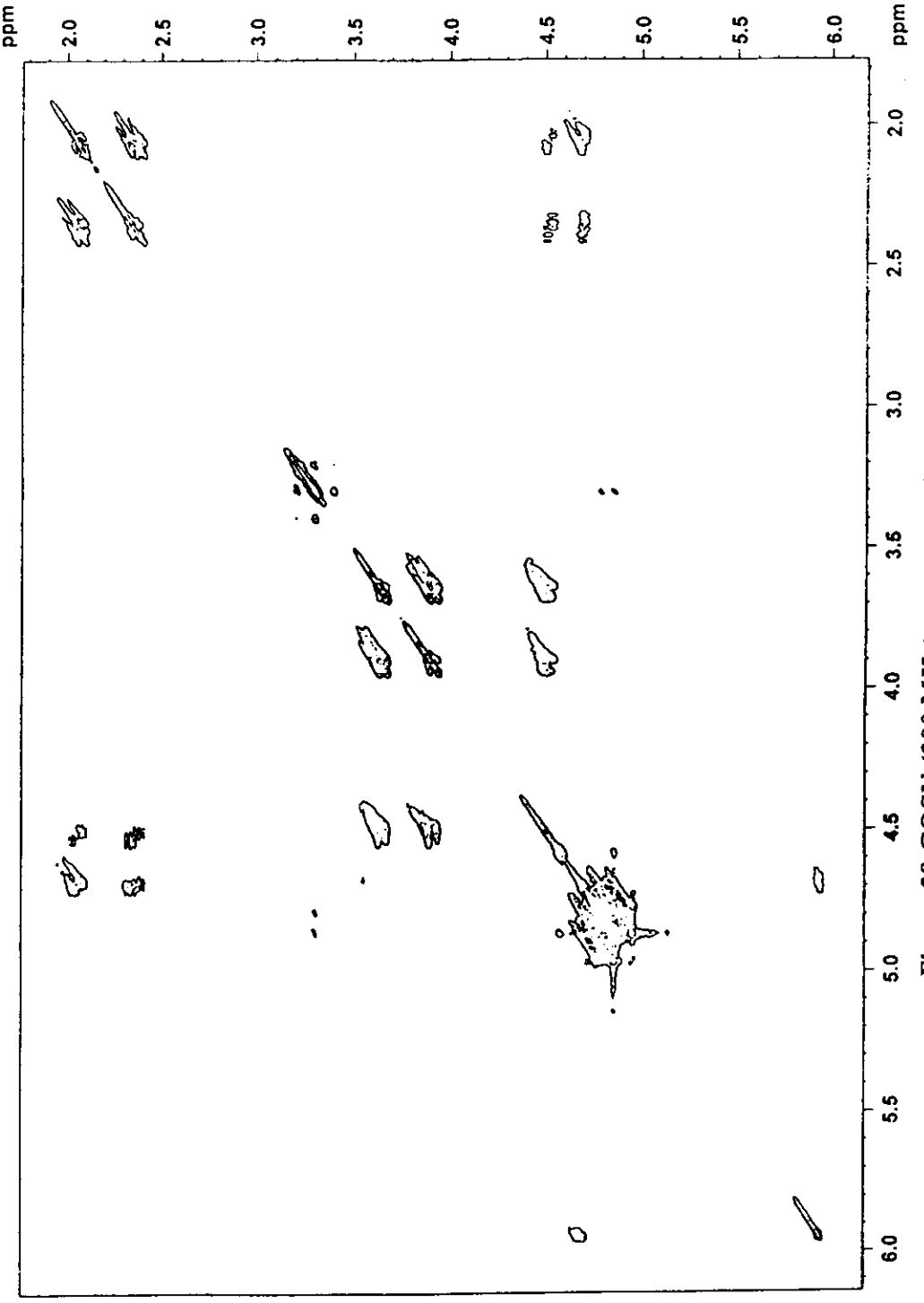


Figure 38 COSY (300 MHz) spectrum of VR-JOY10

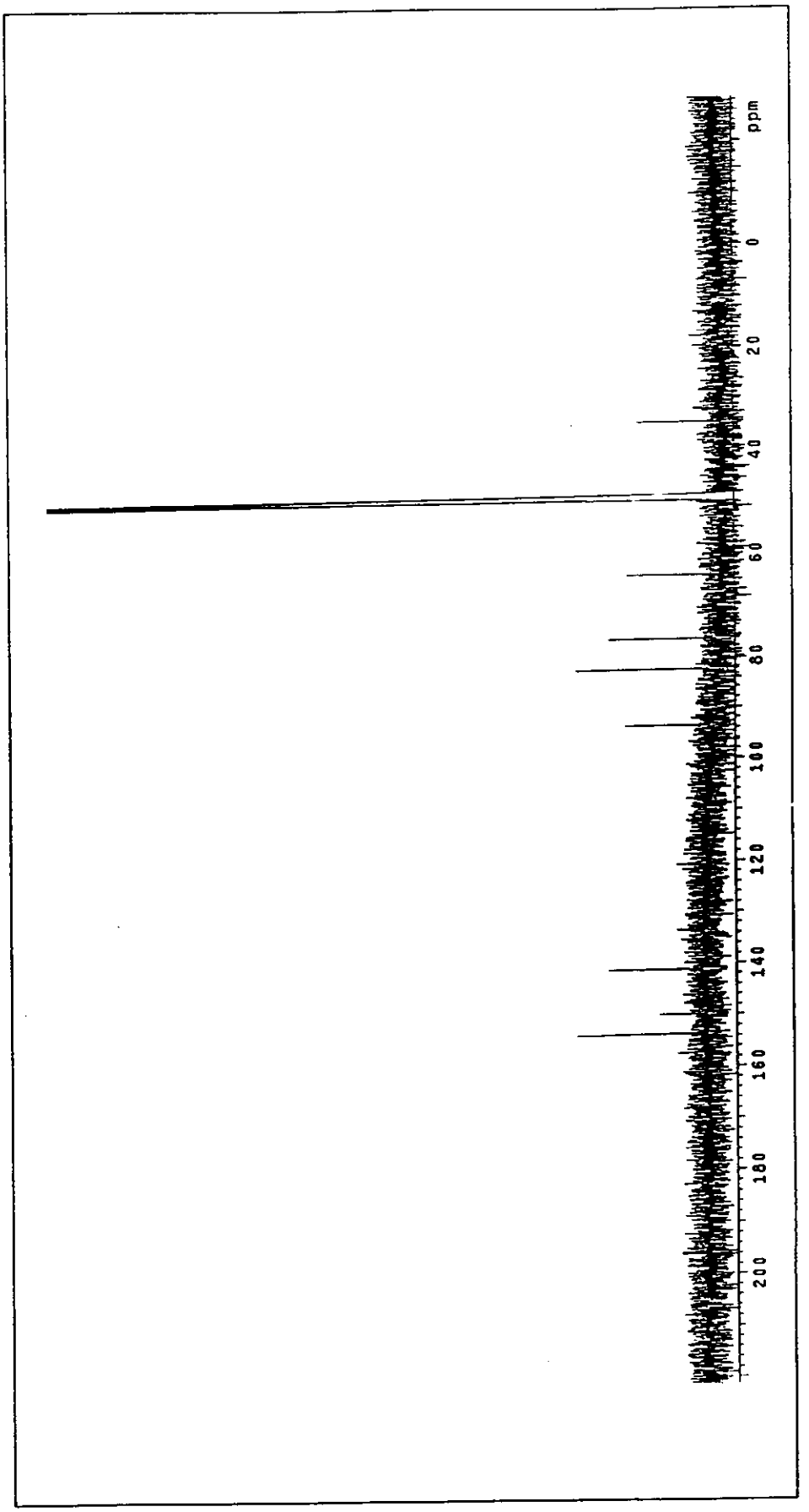


Figure 39 ^{13}C NMR (125 MHz) (CD_3OD) spectrum of VR-JOY10

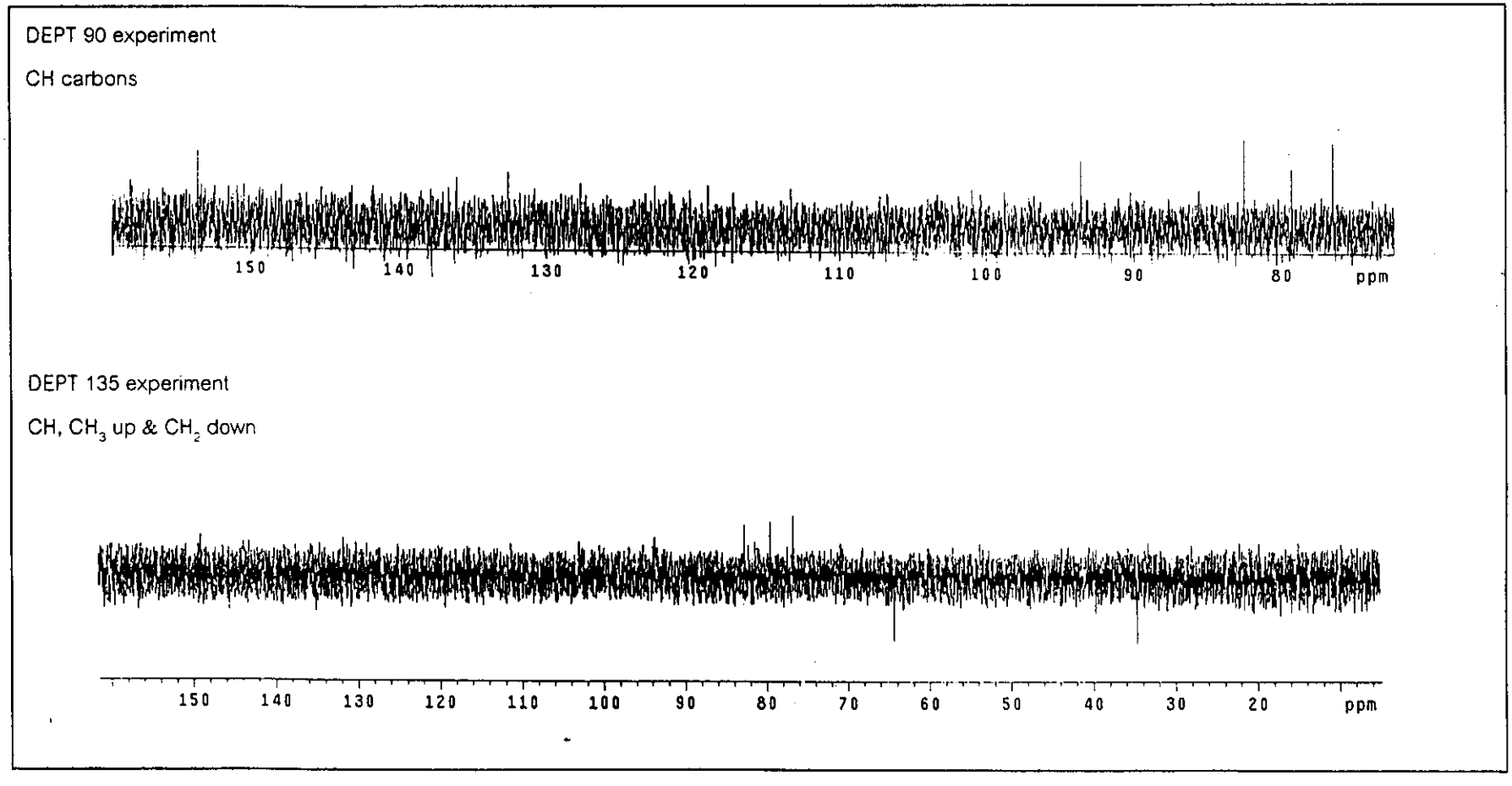


Figure 40 DEPT spectrum of VR-JOY10

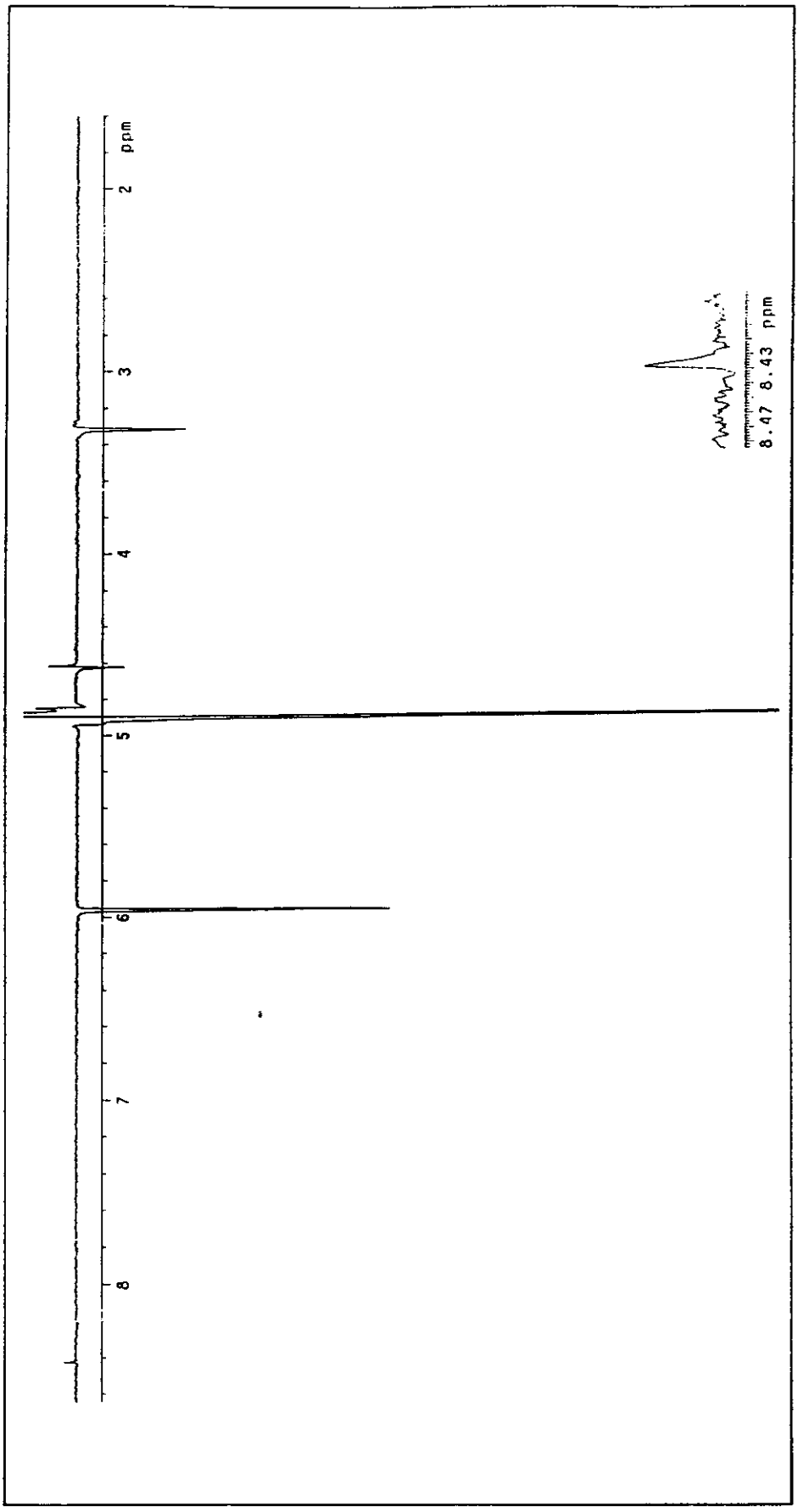


Figure 41 NOEDIFF spectrum of VR-JOY10 after irradiation at δ_H5.95

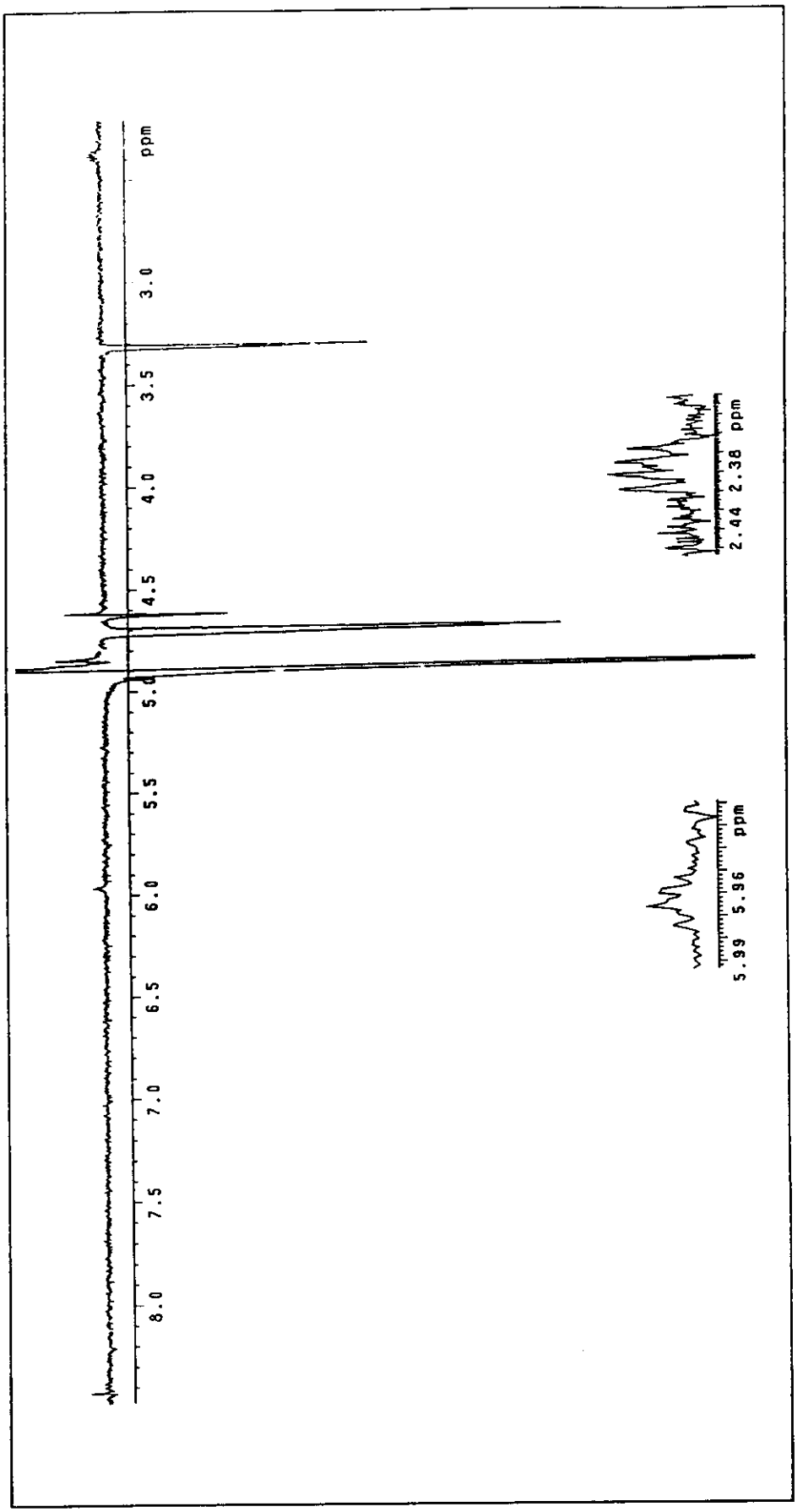


Figure 42 NOEDIFF spectrum of VR-JOY10 after irradiation at δ_H4.71

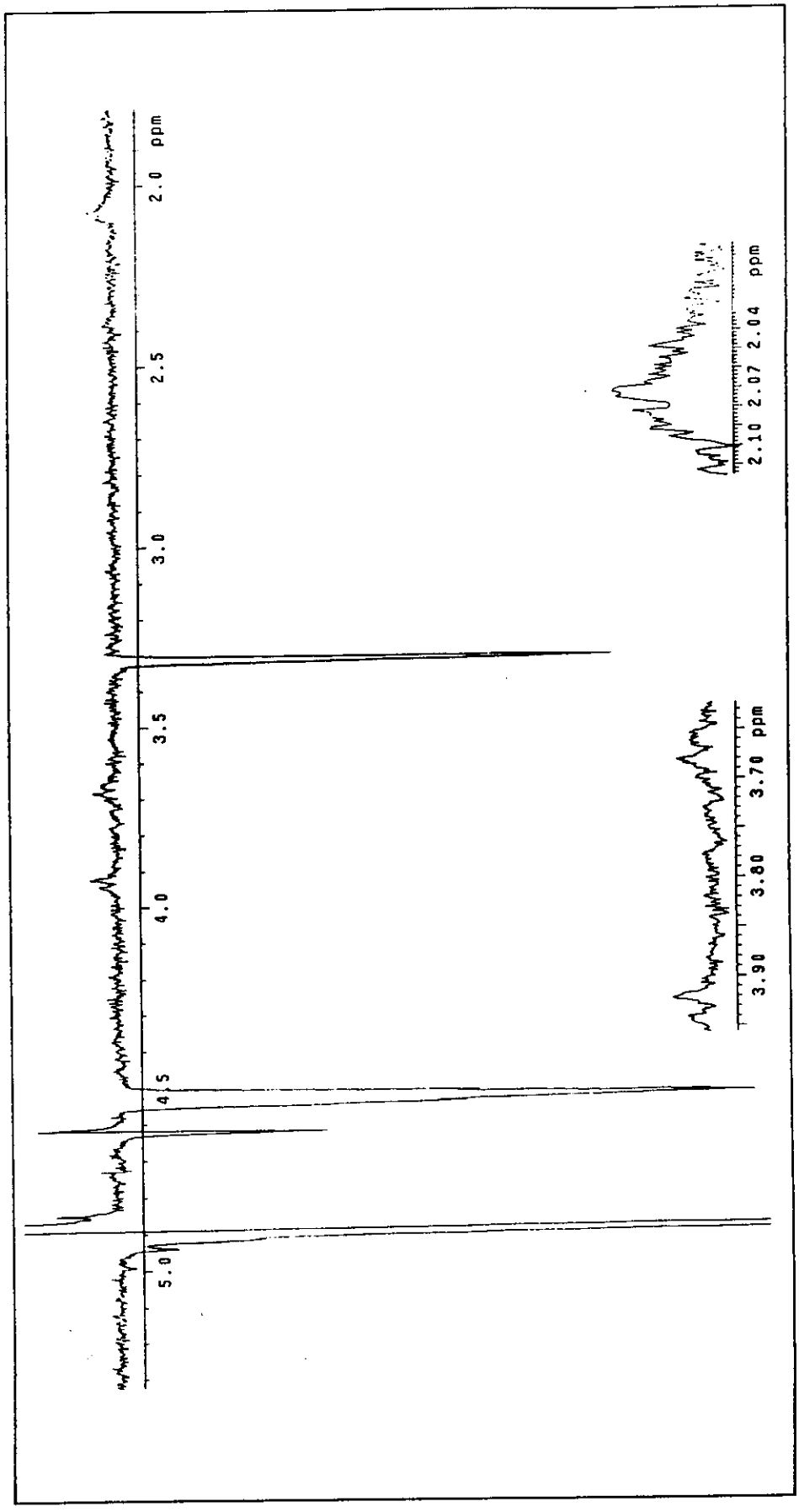


Figure 43 NOEDIFF spectrum of VR-JOY10 after irradiation at δ_H4.52

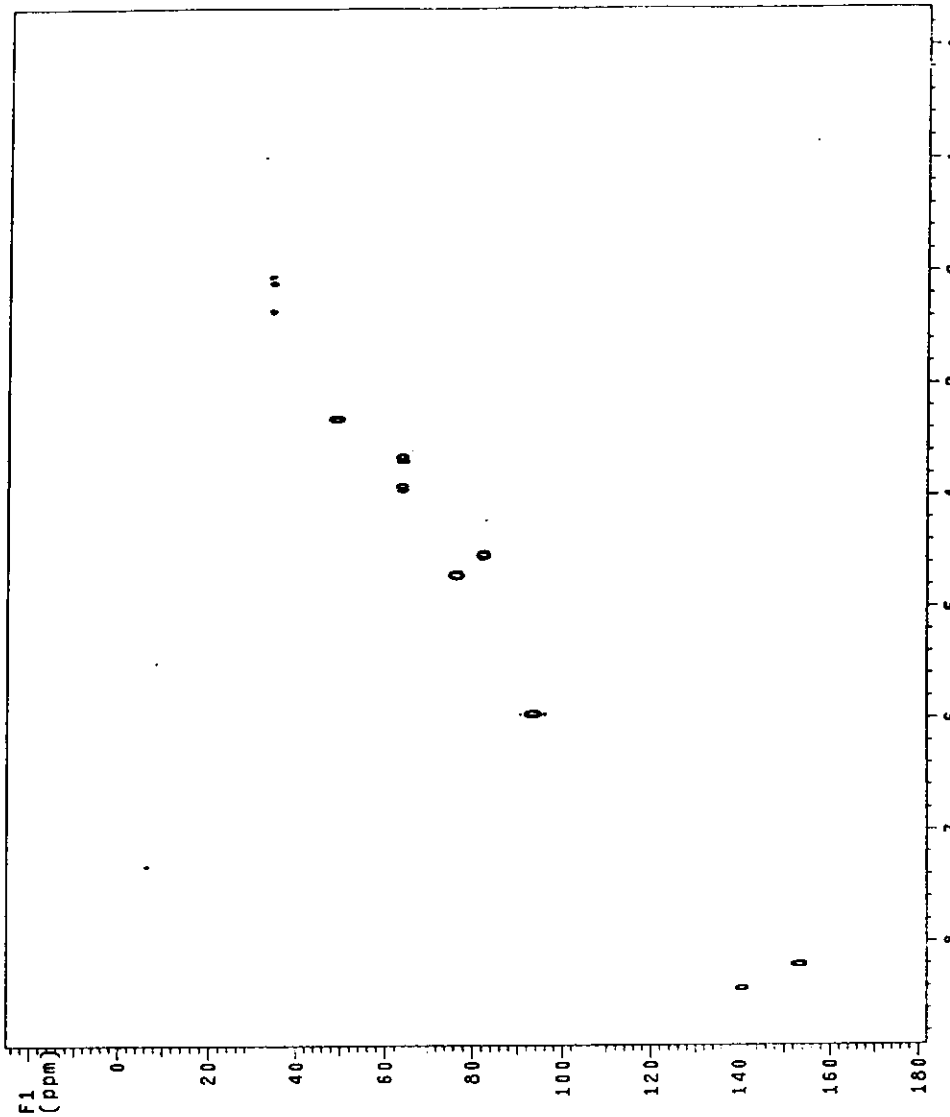


Figure 44 2D HMQC (500 MHz) spectrum of VR-JOY10

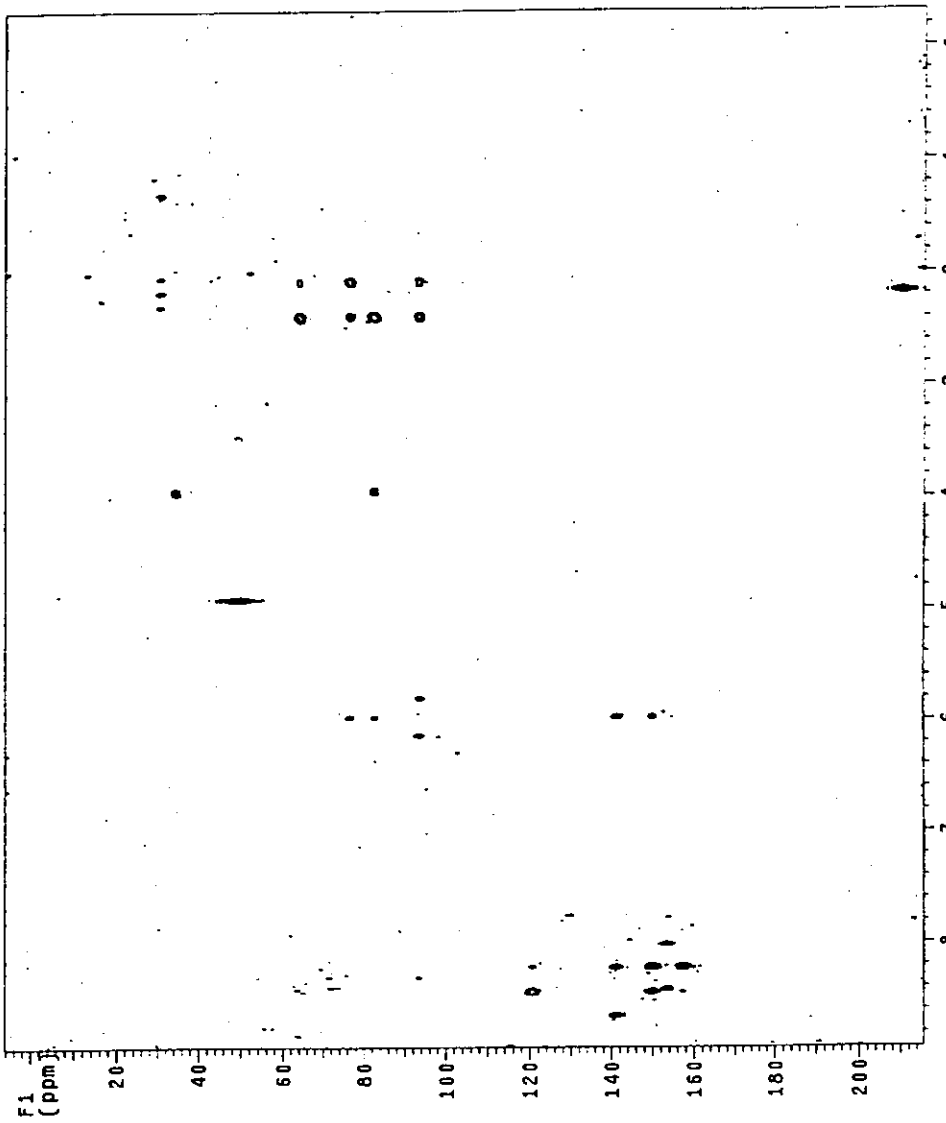


Figure 45 2D HMBC (500 MHz) spectrum of VR-JOY10

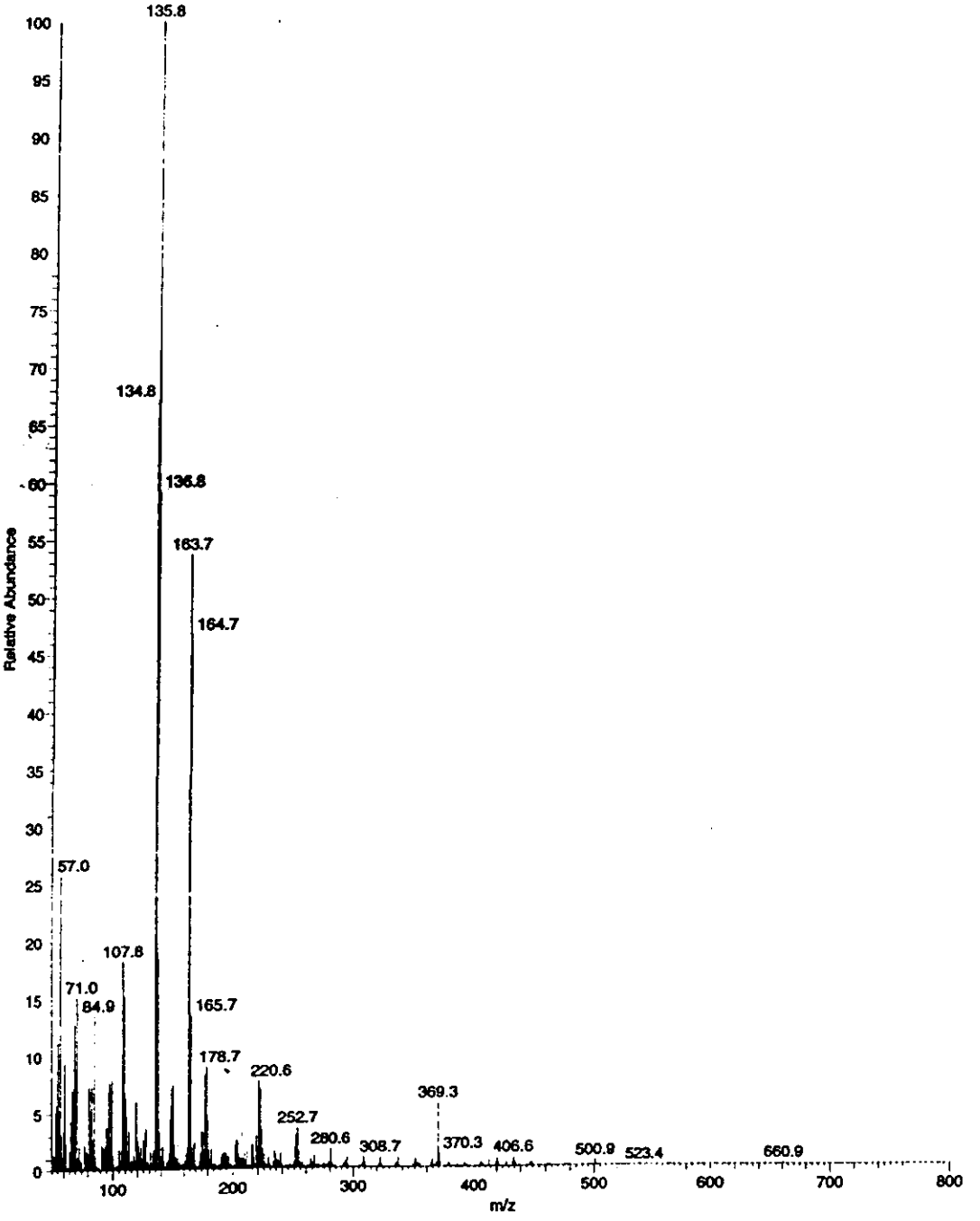


Figure 46 Mass spectrum of VR-JOY10

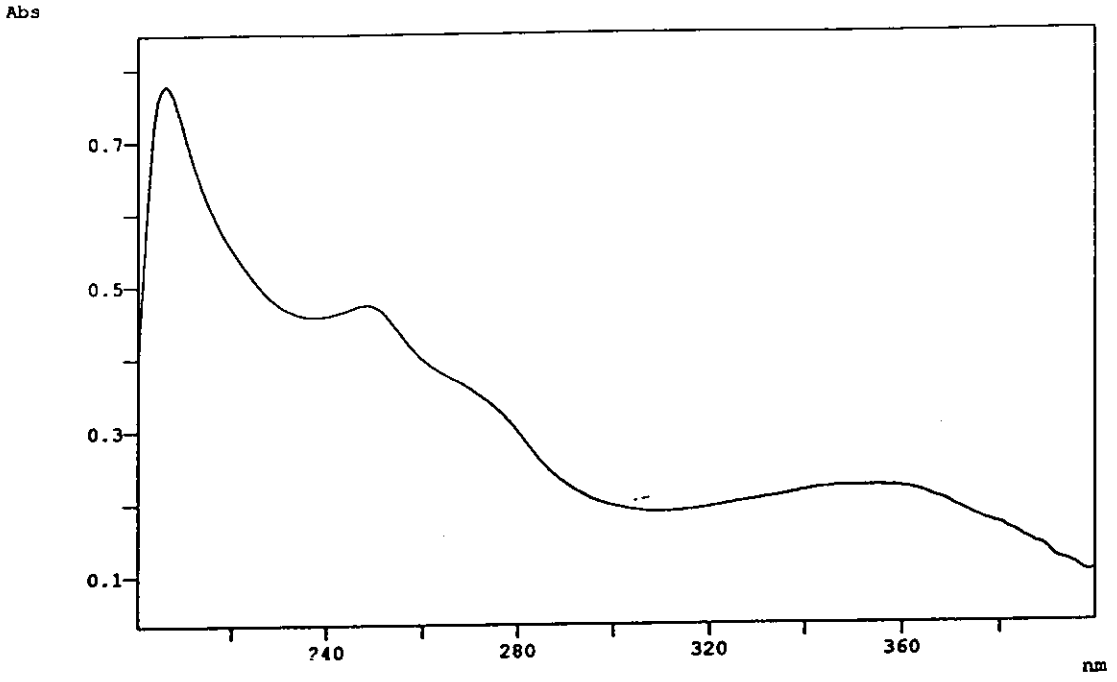


Figure 47 UV (MEOH) spectrum of VR-JOY9

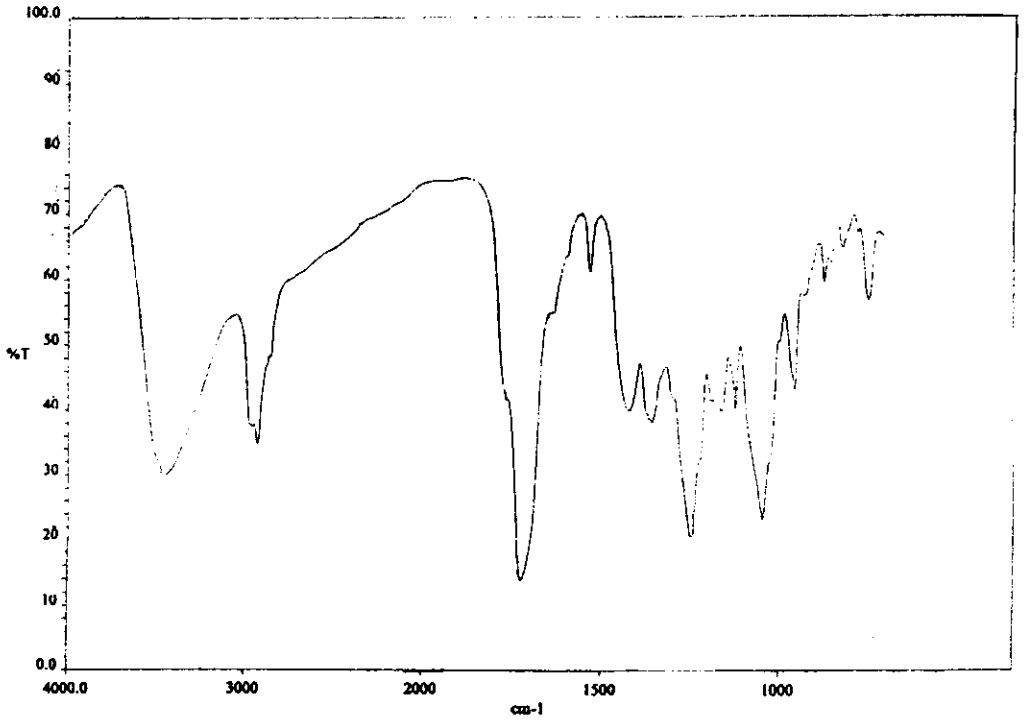


Figure 48 FT-IR (neat) spectrum of VR-JOY9

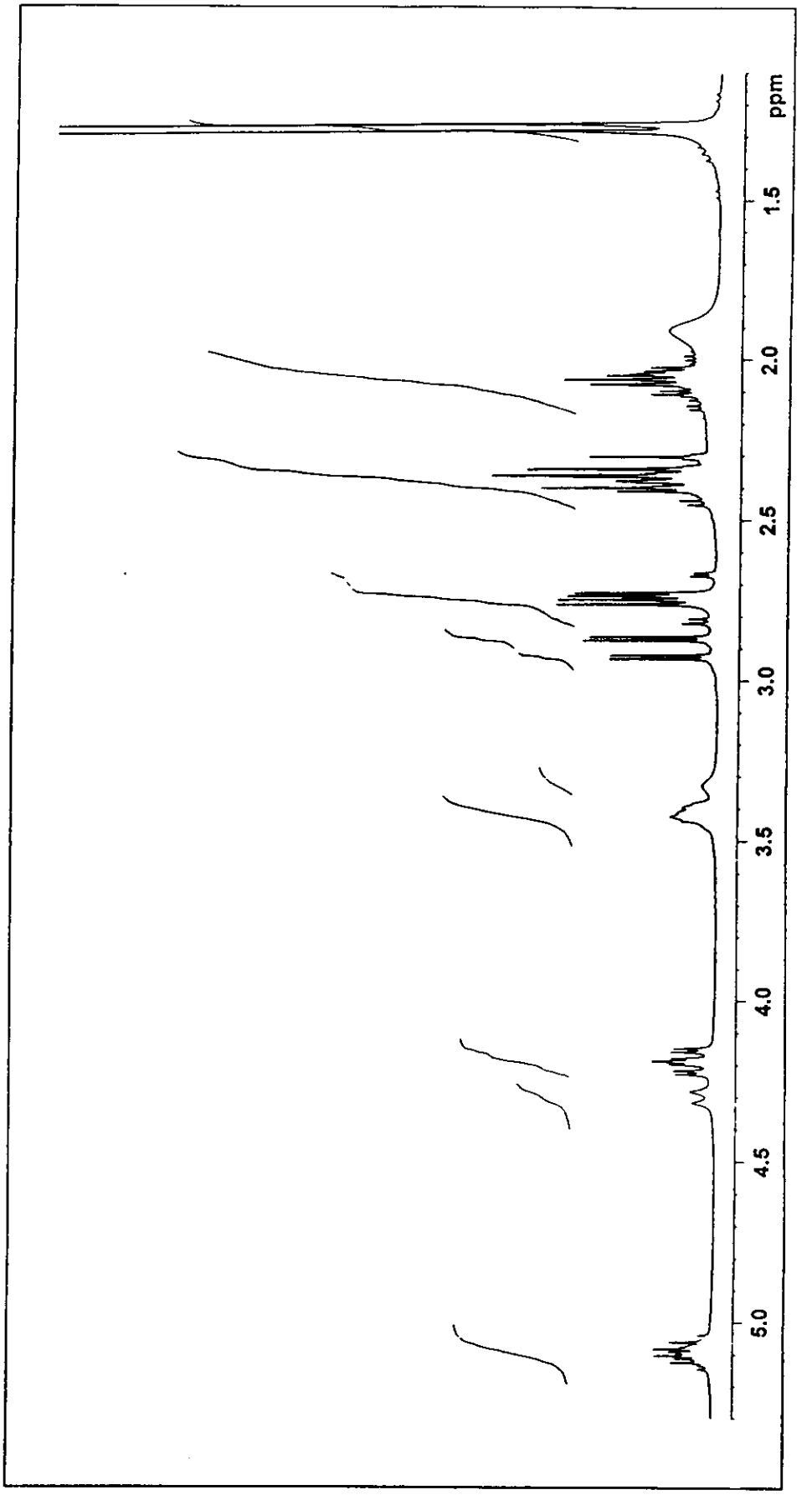


Figure 49 ^{1}H NMR (300 MHz) ($CDCl_3$+CD_3OD) spectrum of VR-JOY9

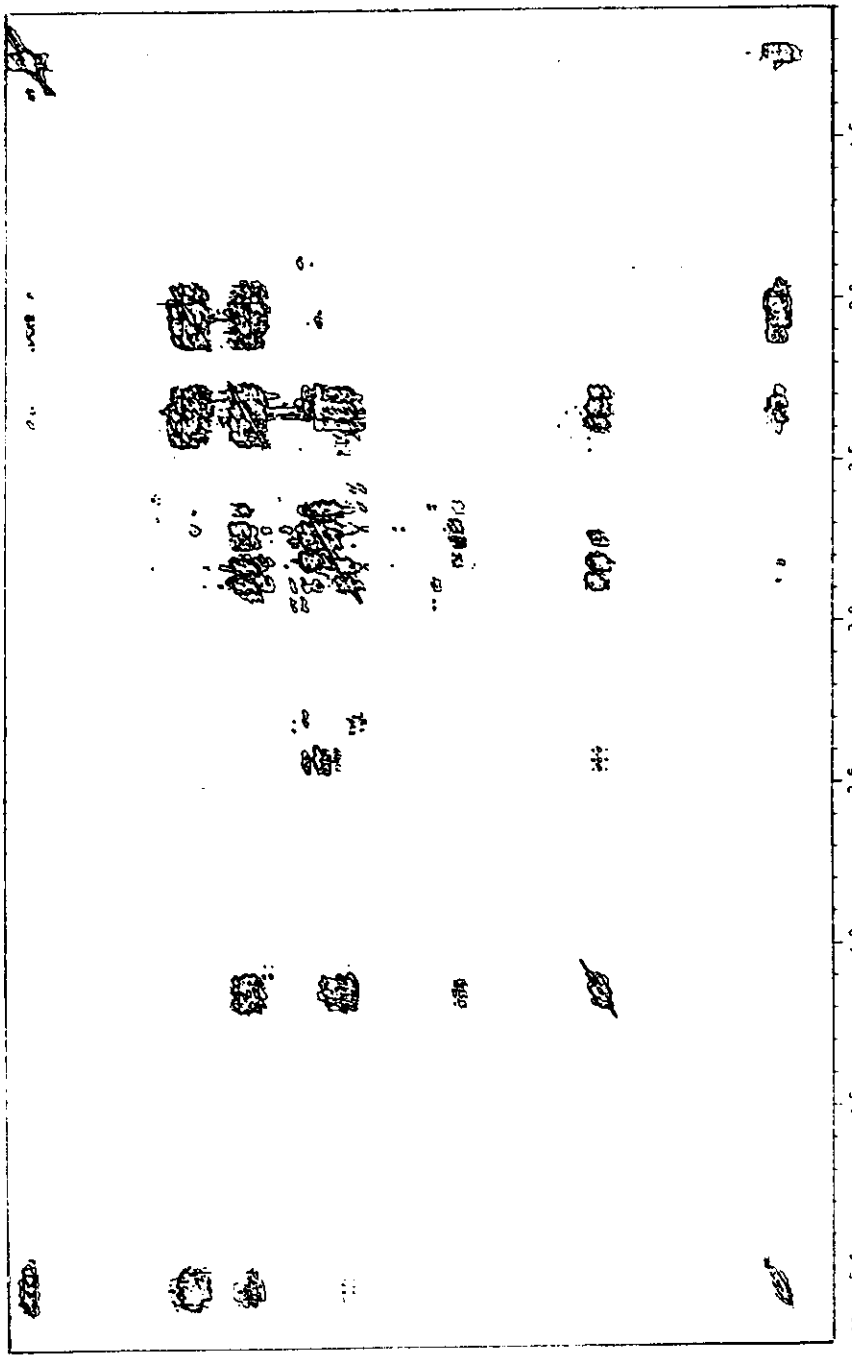


Figure 50 COSY (300 MHz) spectrum of VR-JOY9

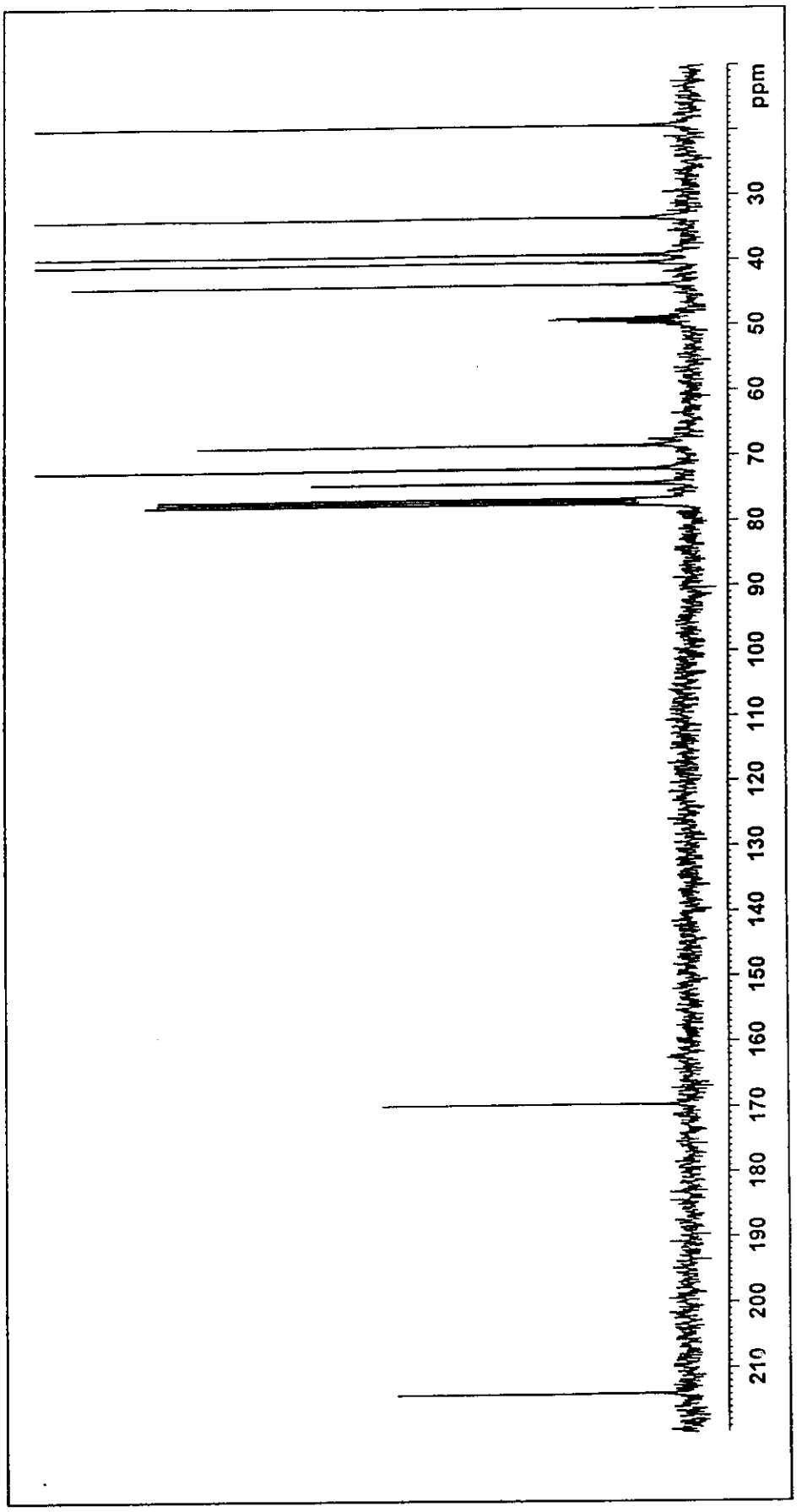


Figure 51 ^{13}C NMR (75 MHz) ($CDCl_3$+CD_3OD) spectrum of VR-JOY9

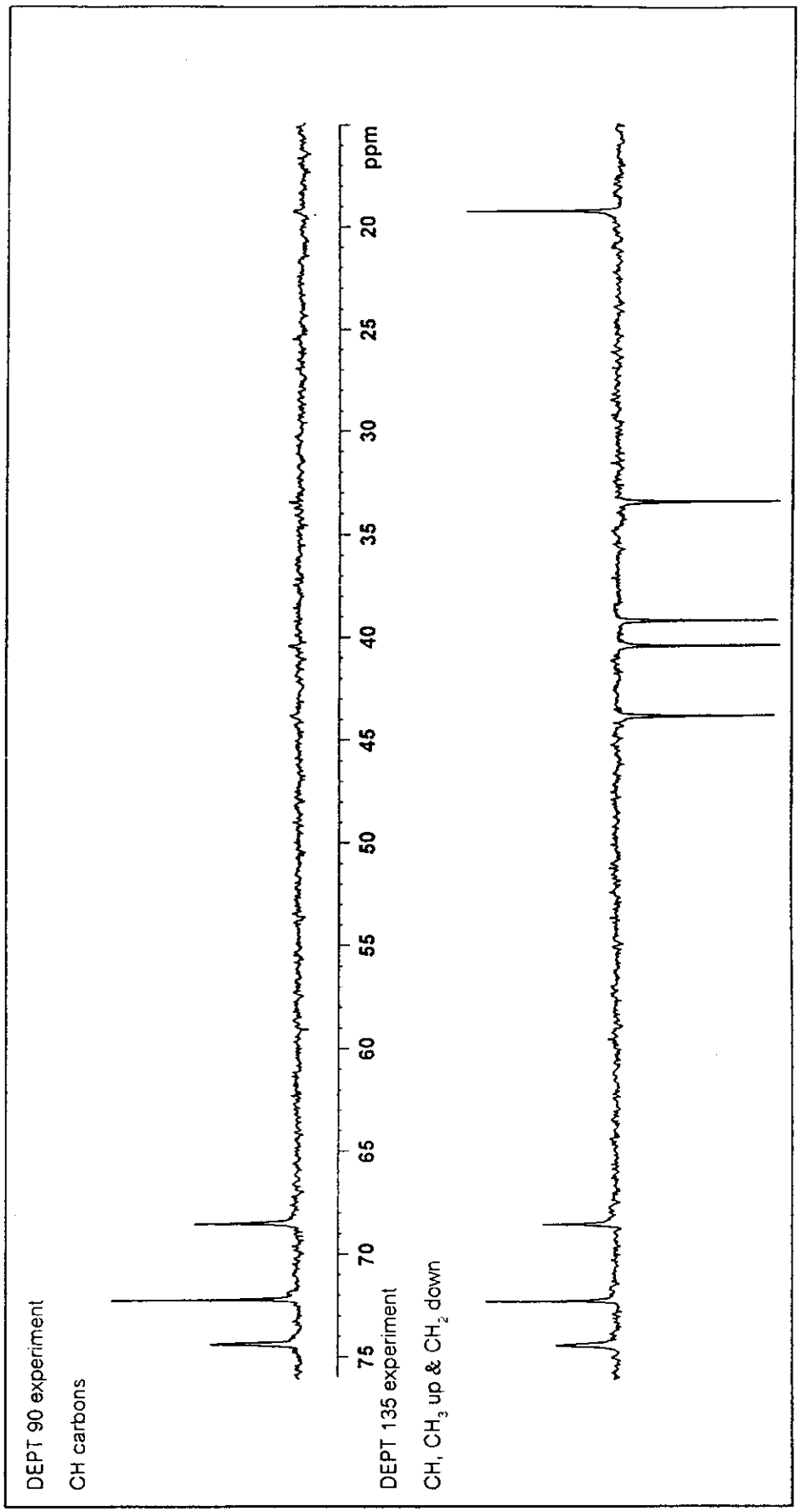


Figure 52 DEPT spectrum of VR-JOY9

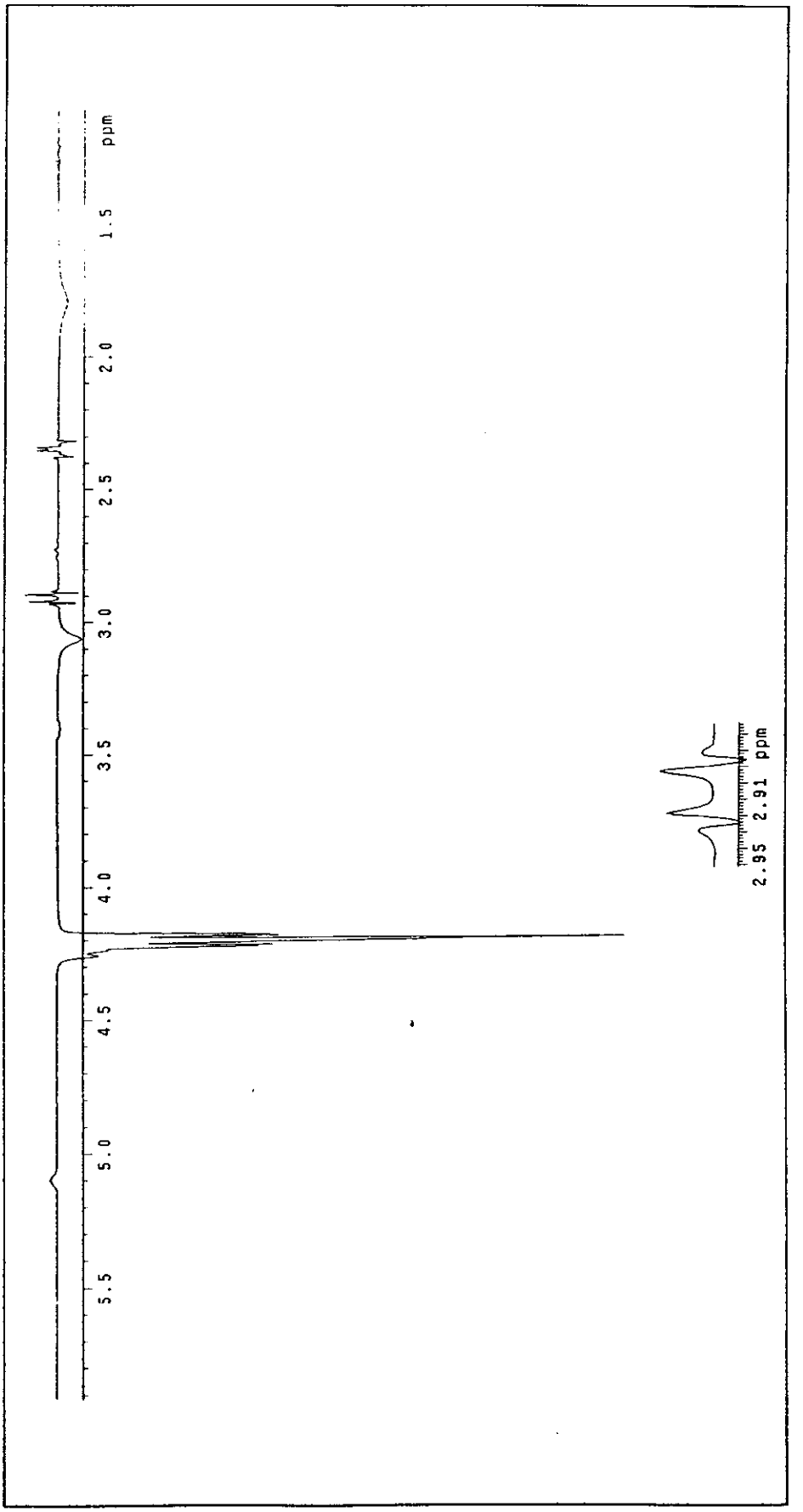


Figure 53 NOEDIFF spectrum of VR-JOY9 after irradiation at δ_H4.17

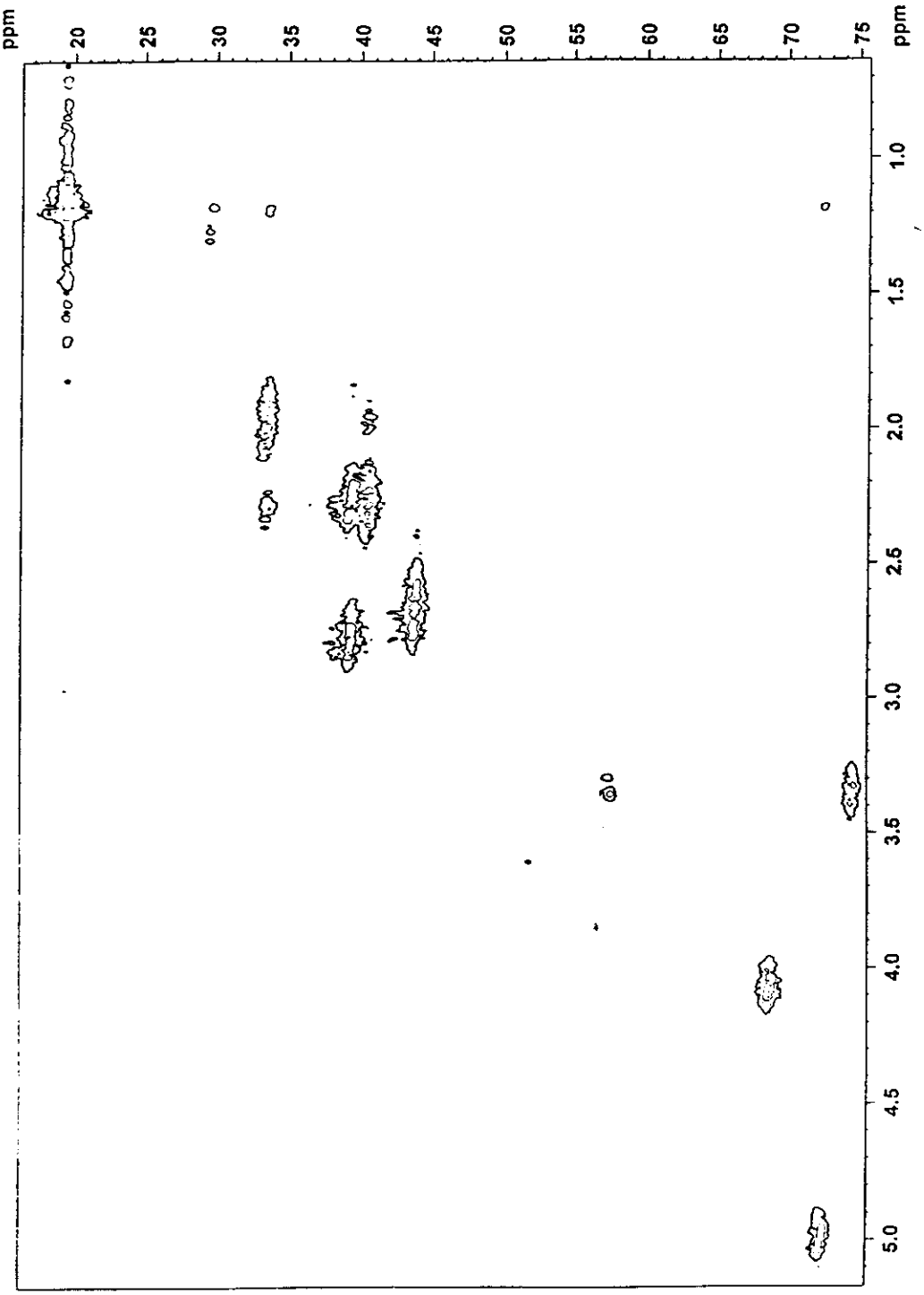


Figure 54 2D HMQC (300 MHz) spectrum of VR-JOY9

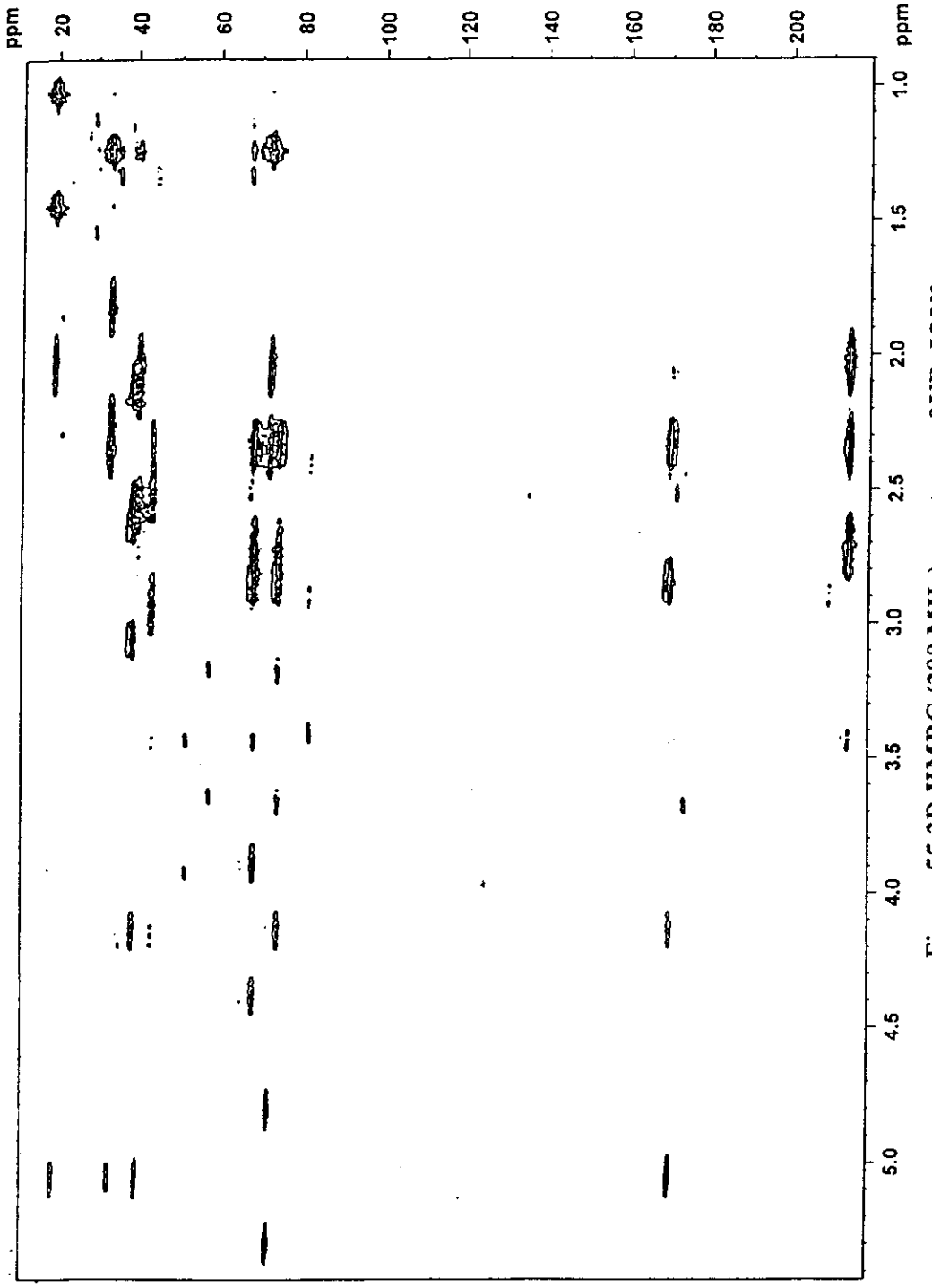


Figure 55 2D HMBC (300 MHz) spectrum of VR-JOY9

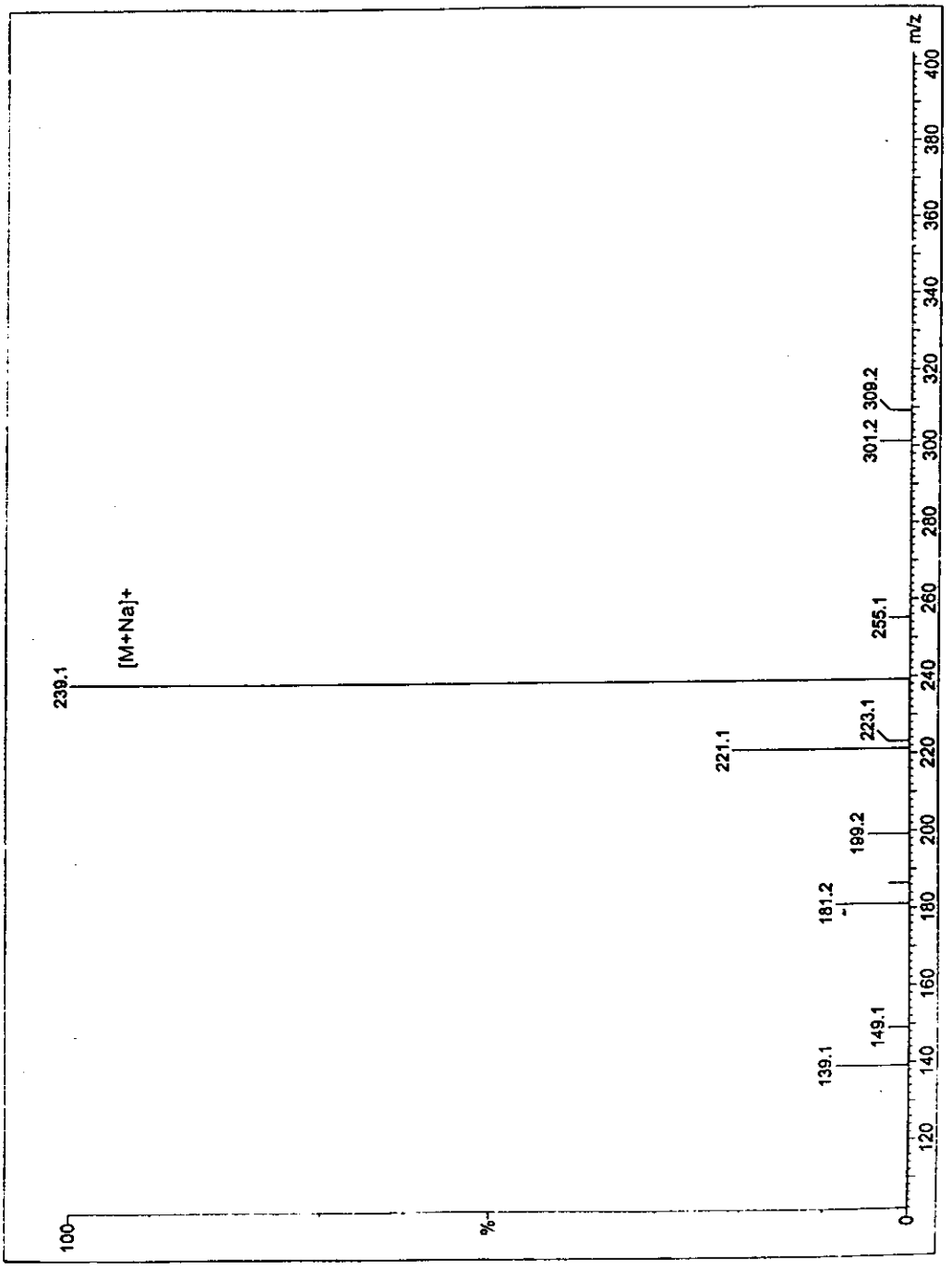


Figure 56 Mass spectrum of VR-JOY9

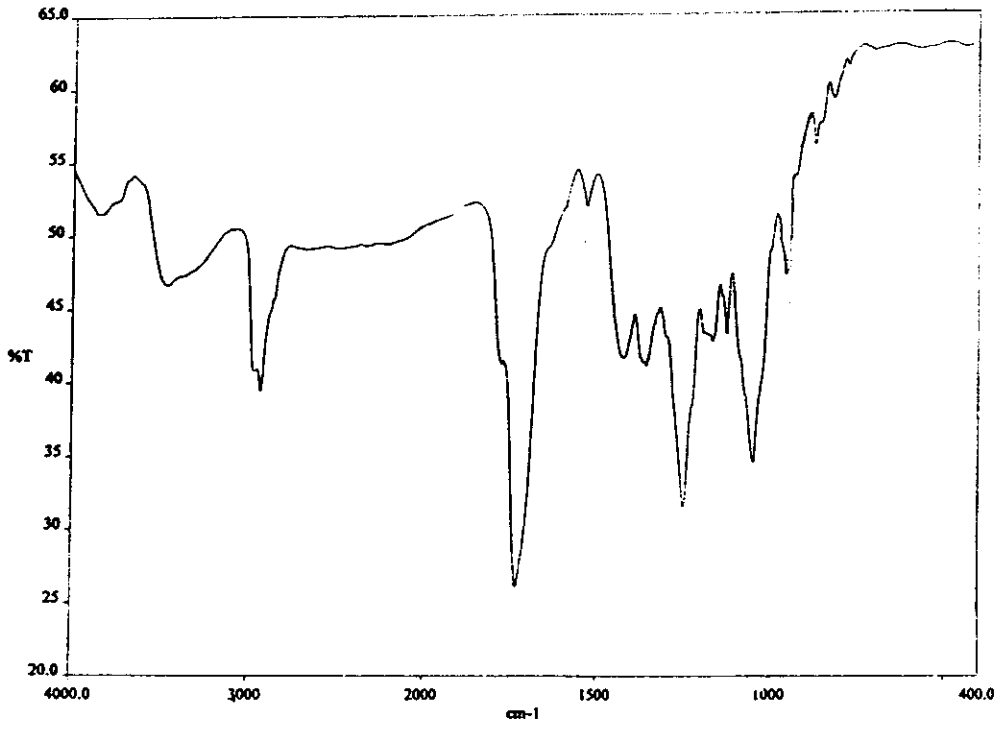


Figure 57 FT-IR (neat) spectrum of VR-JOY12

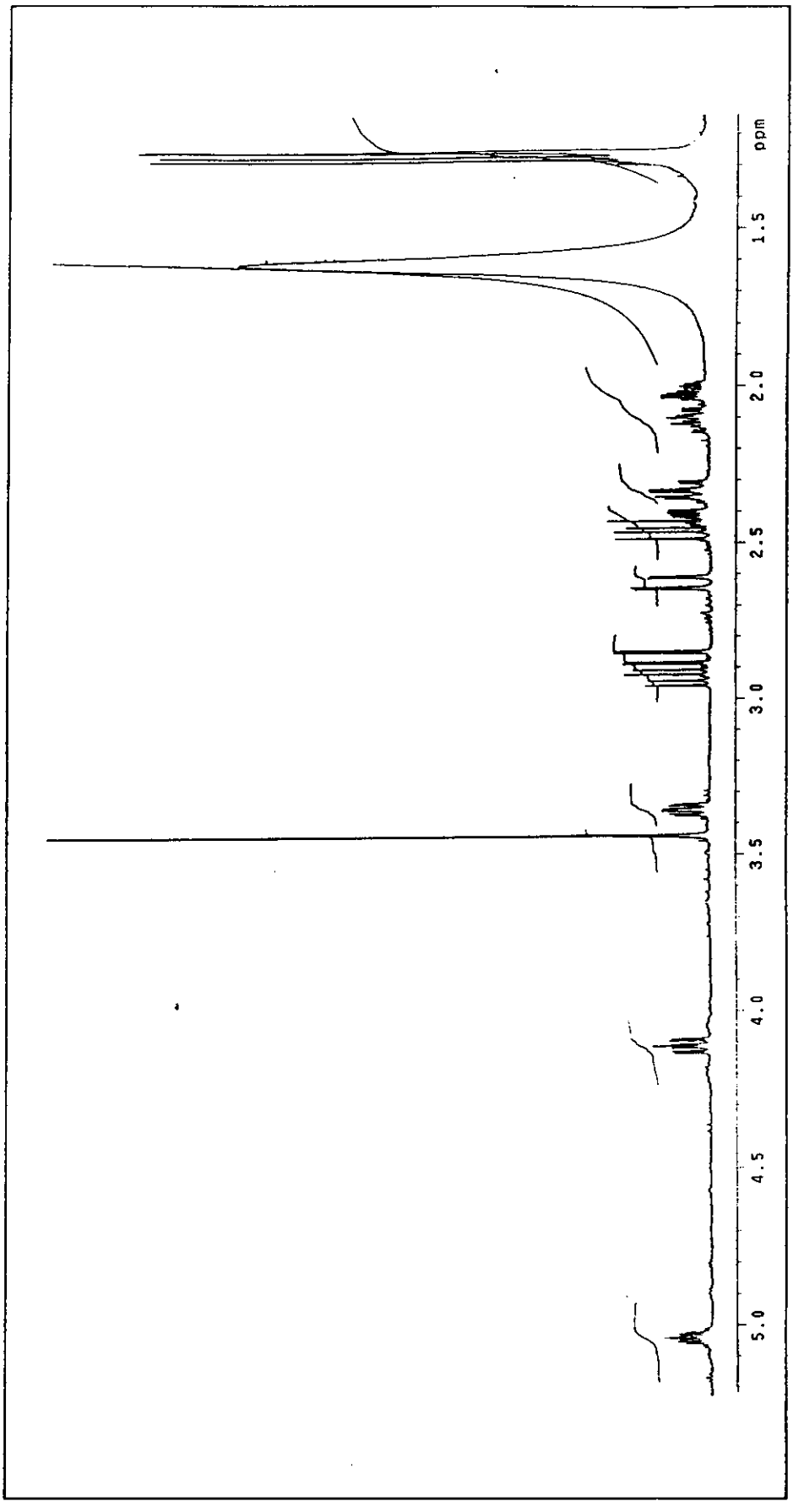


Figure 58 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of VR-JOY12

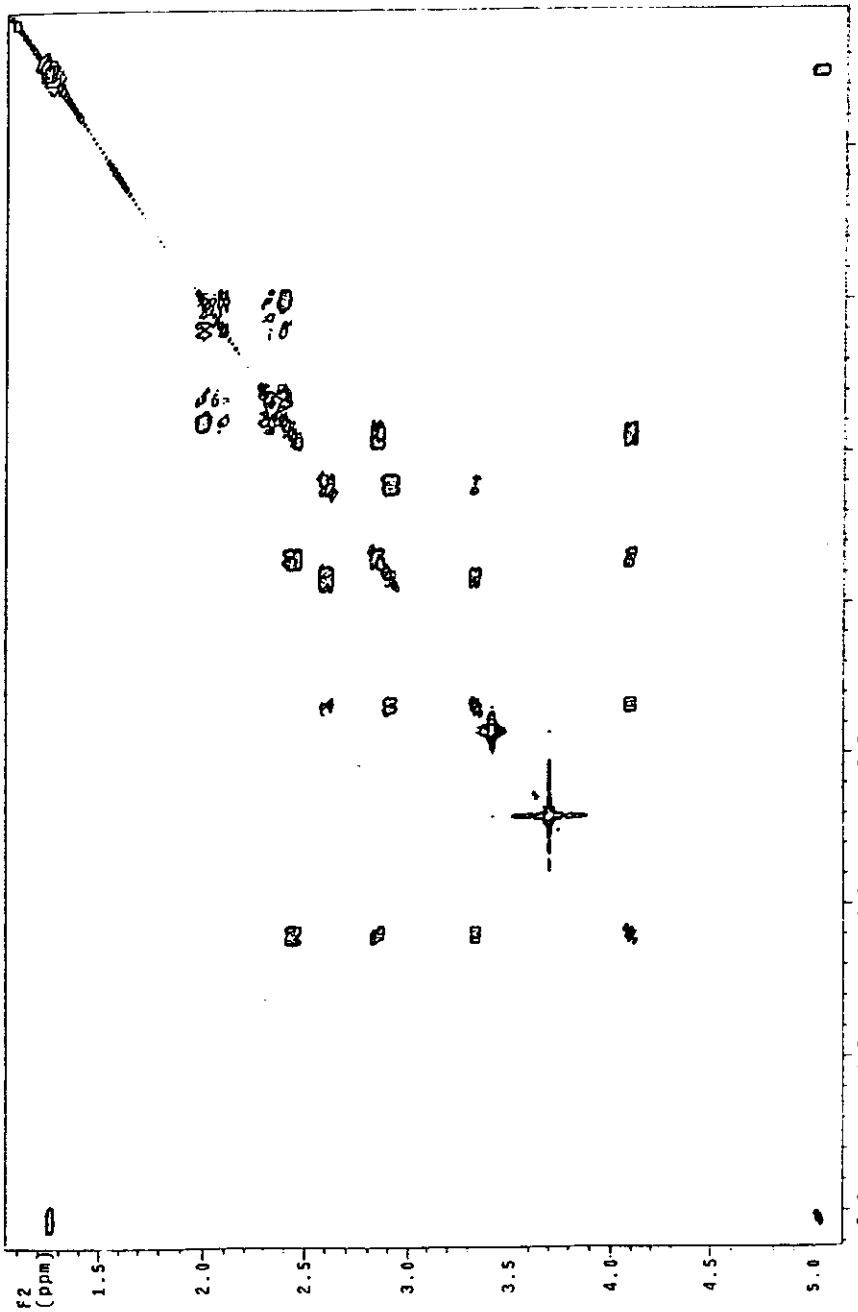


Figure 59 COSY (500 MHz) spectrum of VR-JOY12

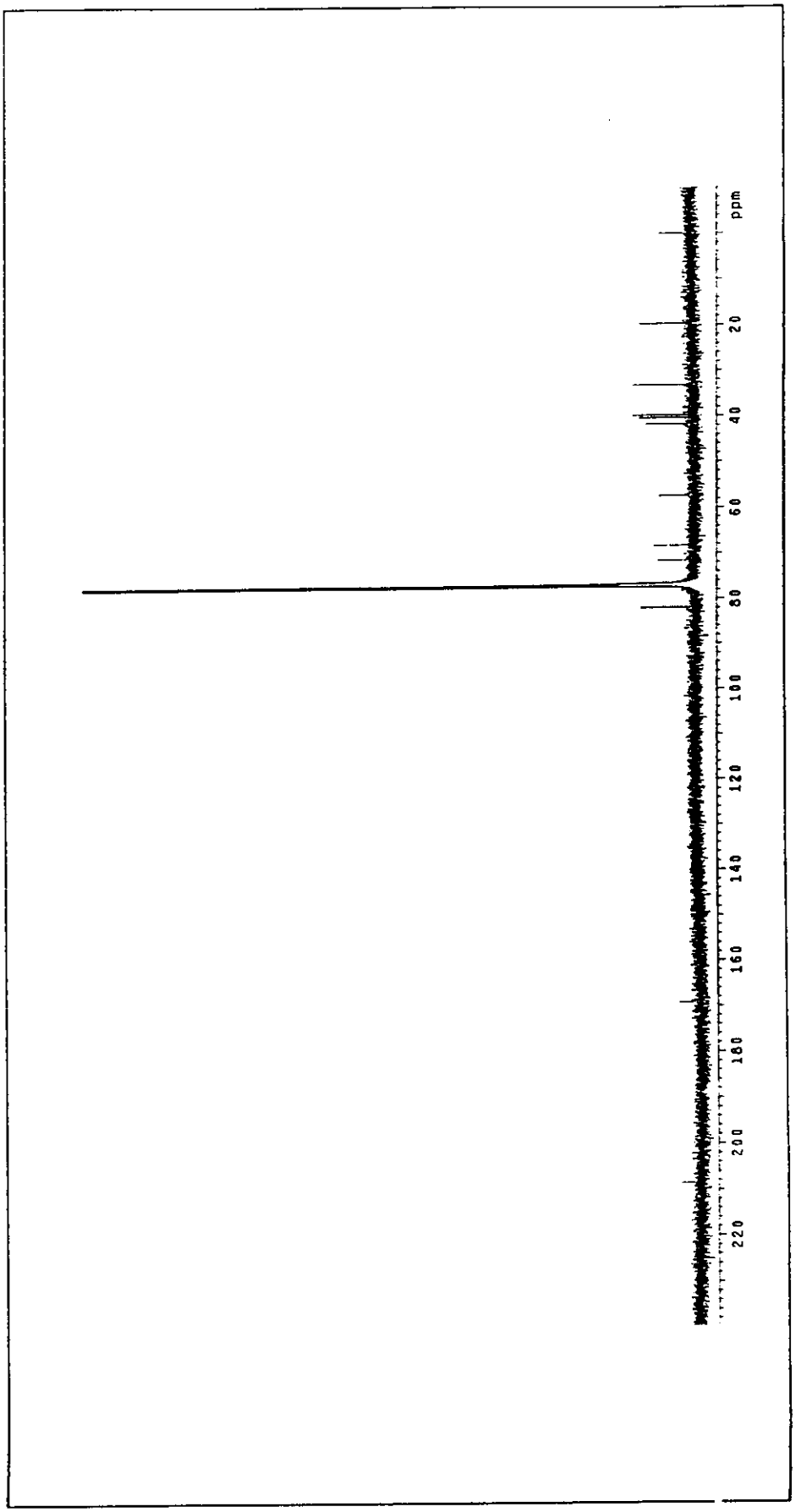
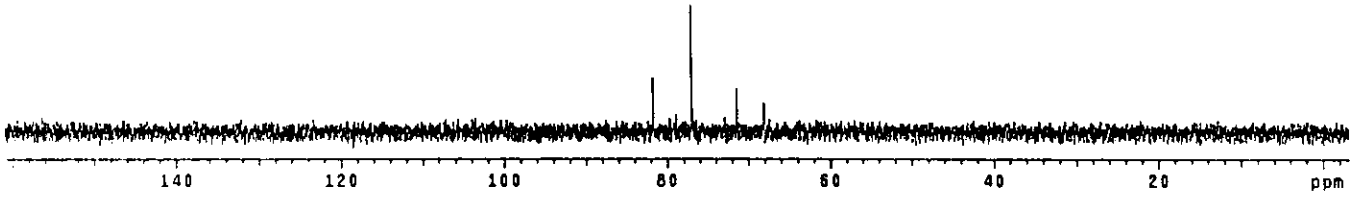


Figure 60 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of VR-JOY12

DEPT 90 experiment

CH carbons



DEPT 135 experiment

CH, CH_3 up & CH_2 down

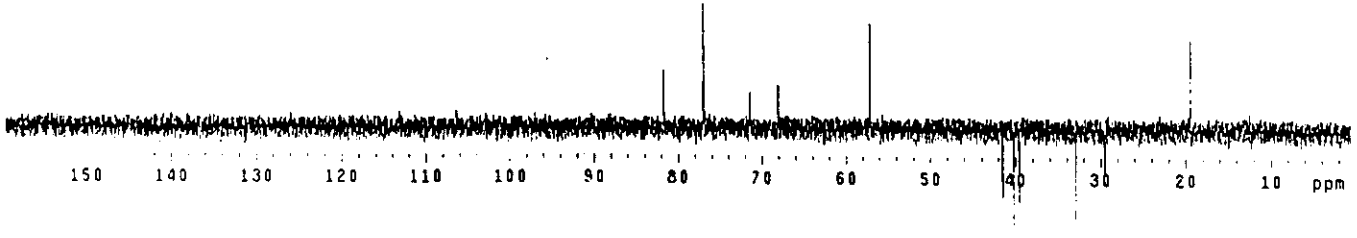


Figure 61 DEPT spectrum of VR-JOY12

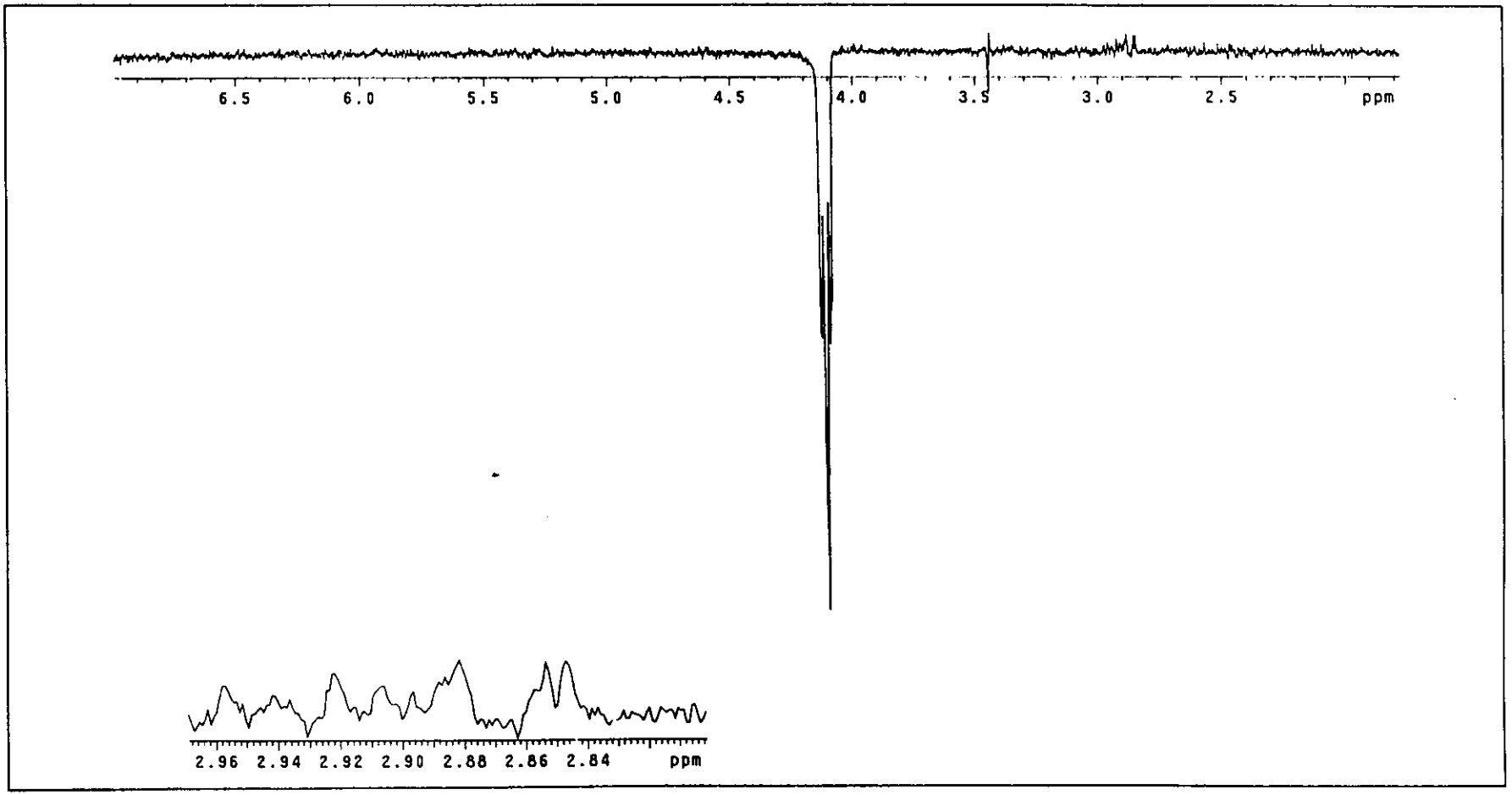


Figure 62 NOEDIFF spectrum of VR-JOY12 after irradiation at δ_H4.11

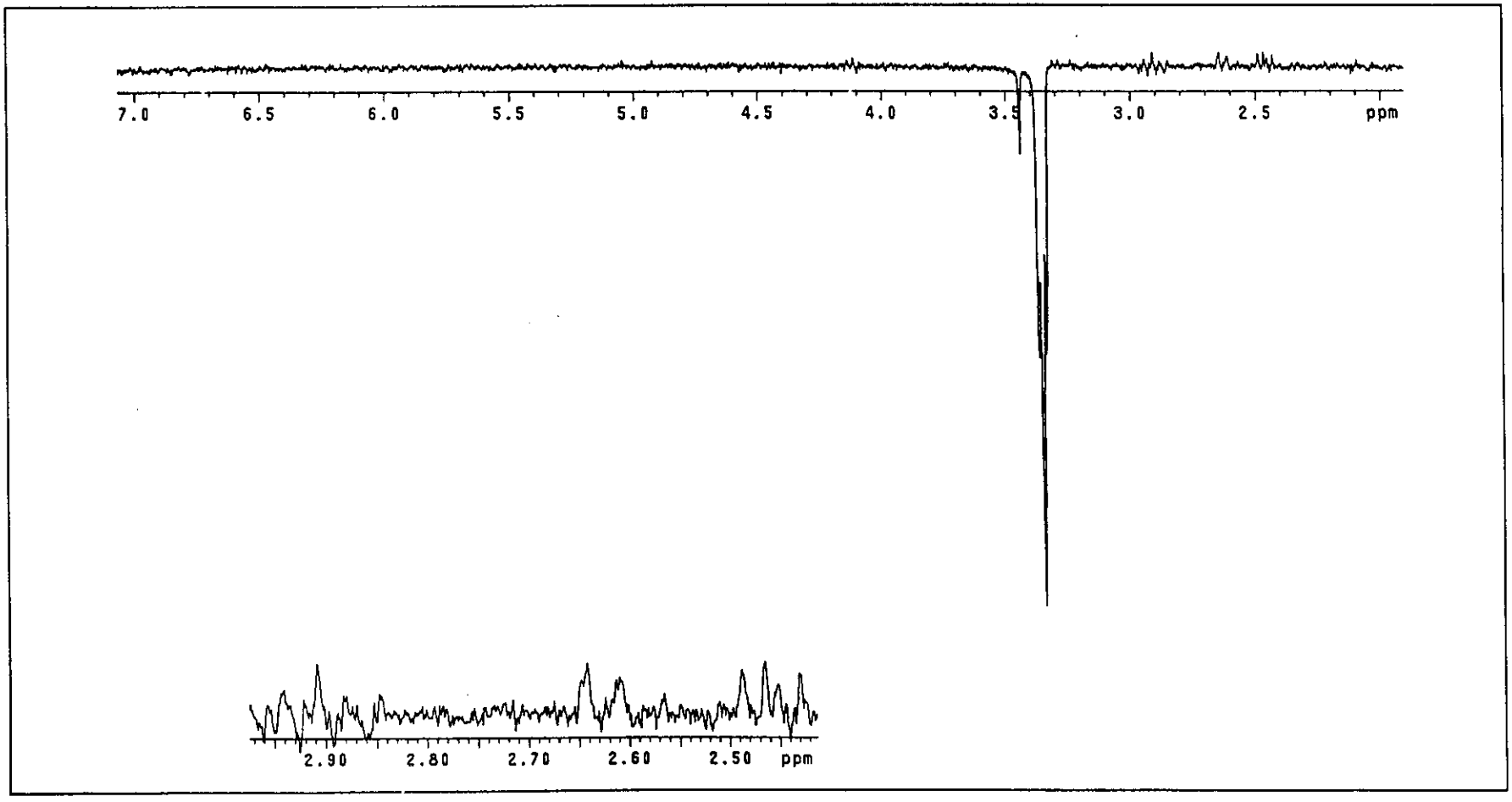


Figure 63 NOEDIFF spectrum of VR-JOY12 after irradiation at δ_H 3.35

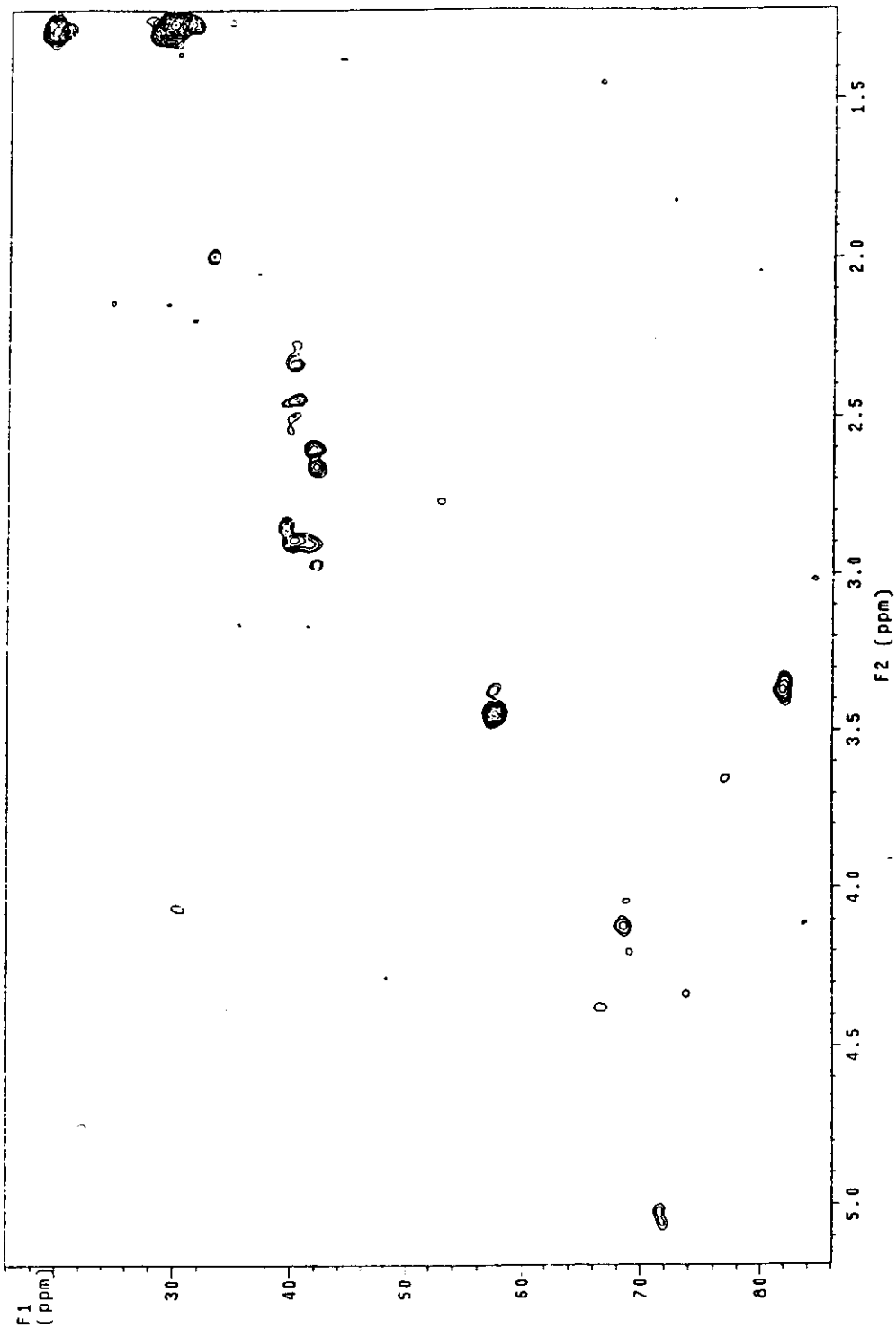


Figure 64 2D HMQC (500 MHz) spectrum of VR-JOY12

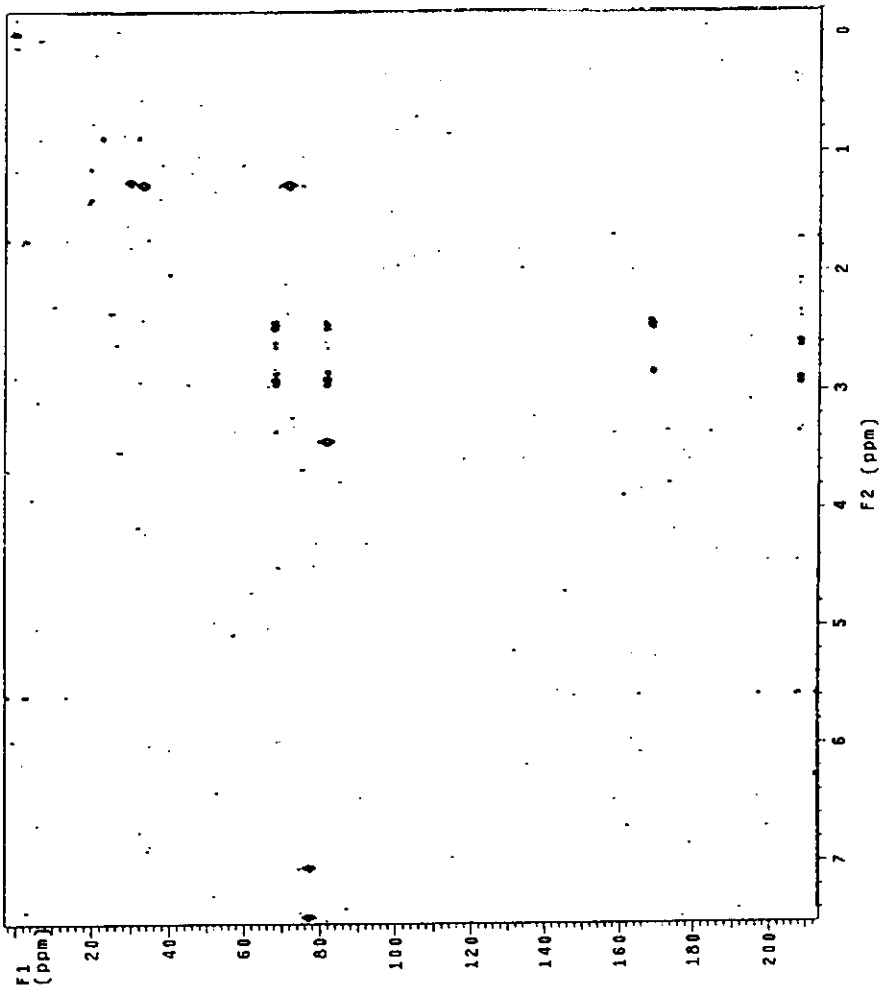


Figure 65 2D HMBC (500 MHz) spectrum of VR-JOY12

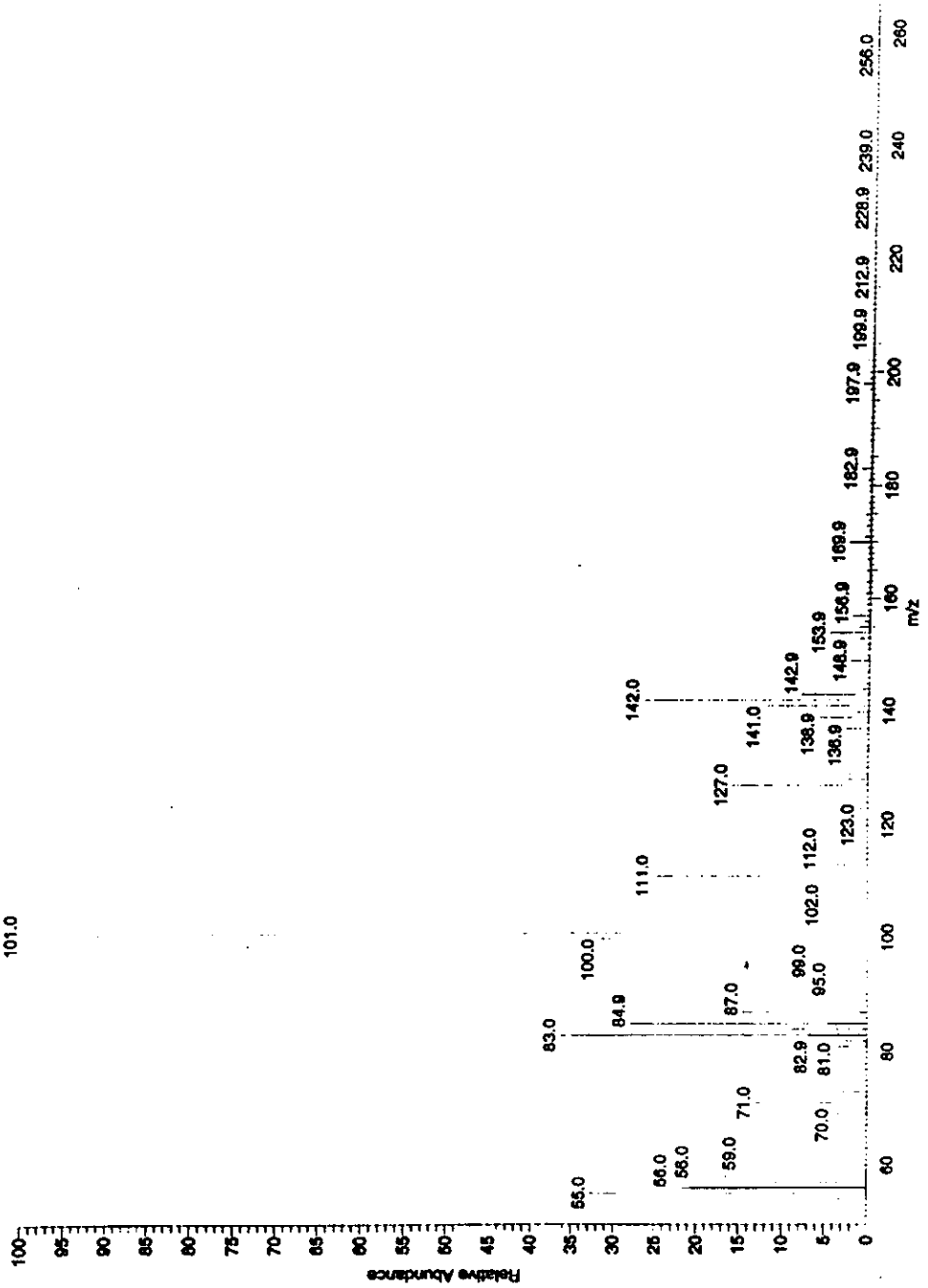


Figure 66 Mass spectrum of VR-JOY12

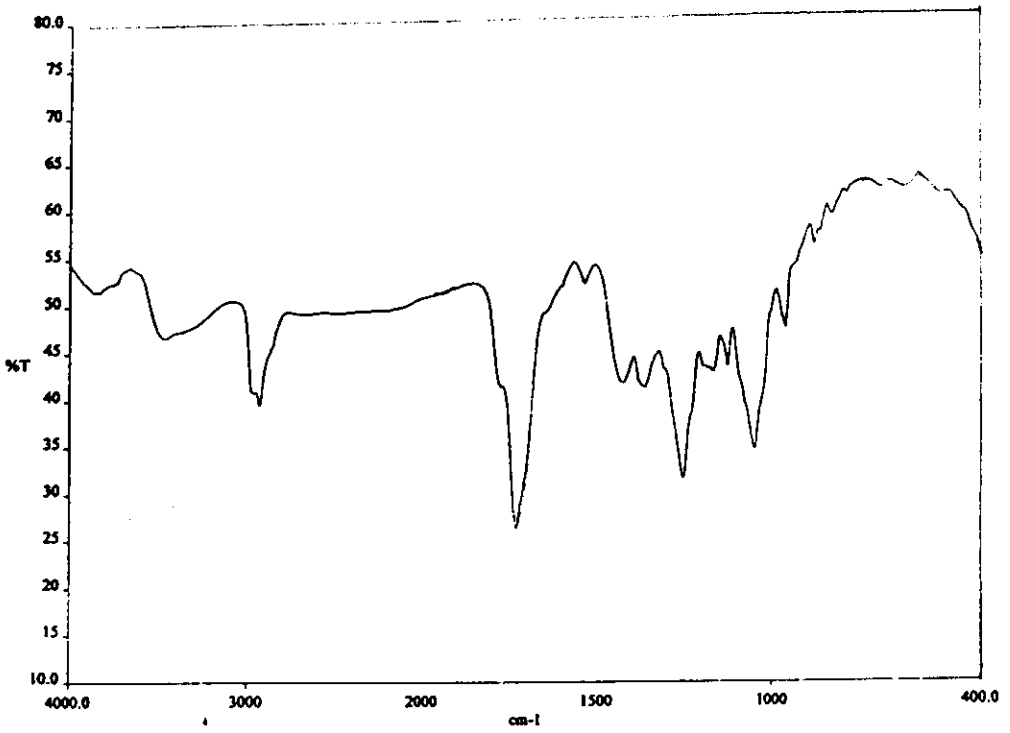


Figure 67 FT-IR (neat) spectrum of VR-JOY13

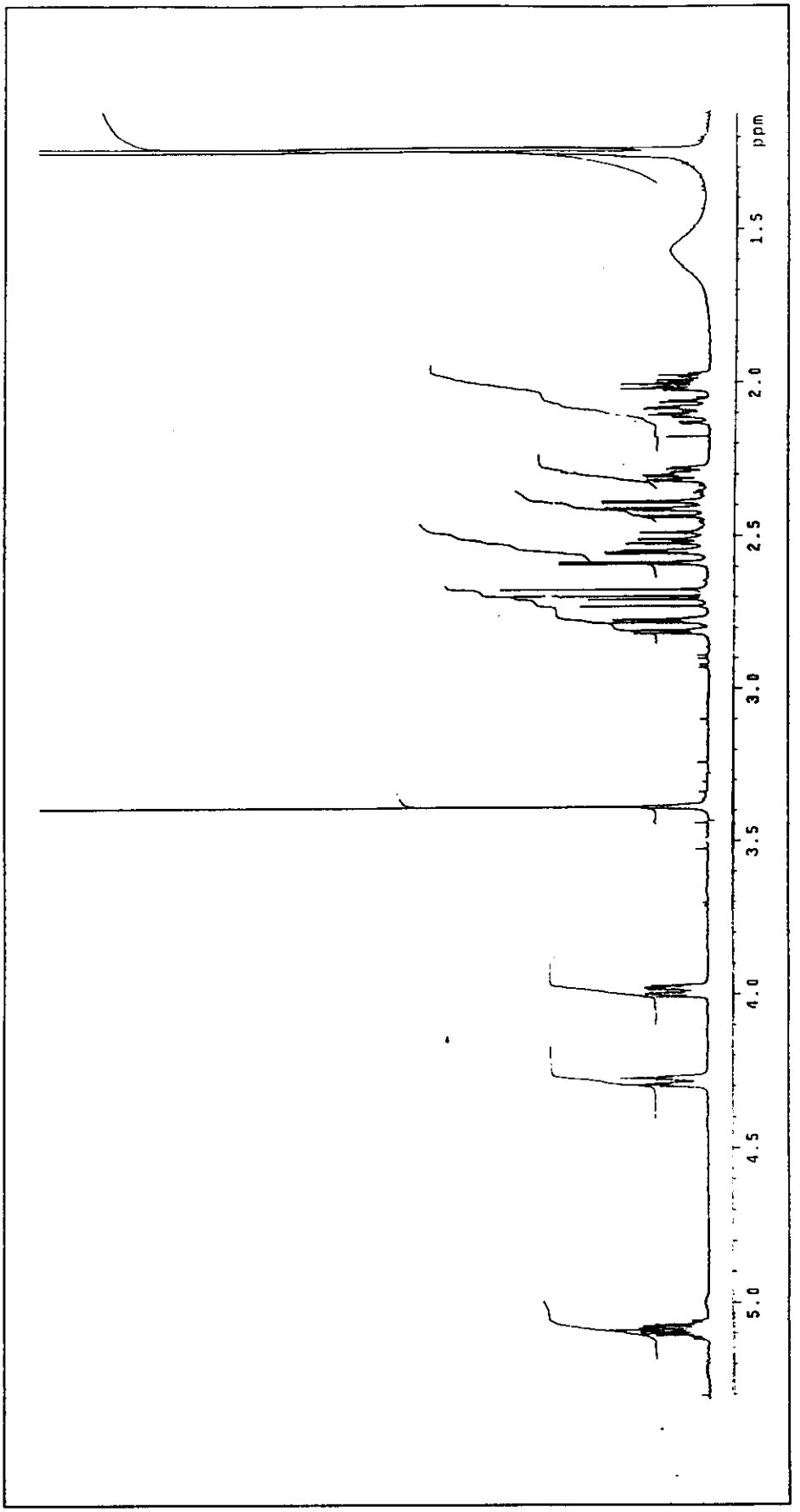


Figure 68 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of VR-JOY13

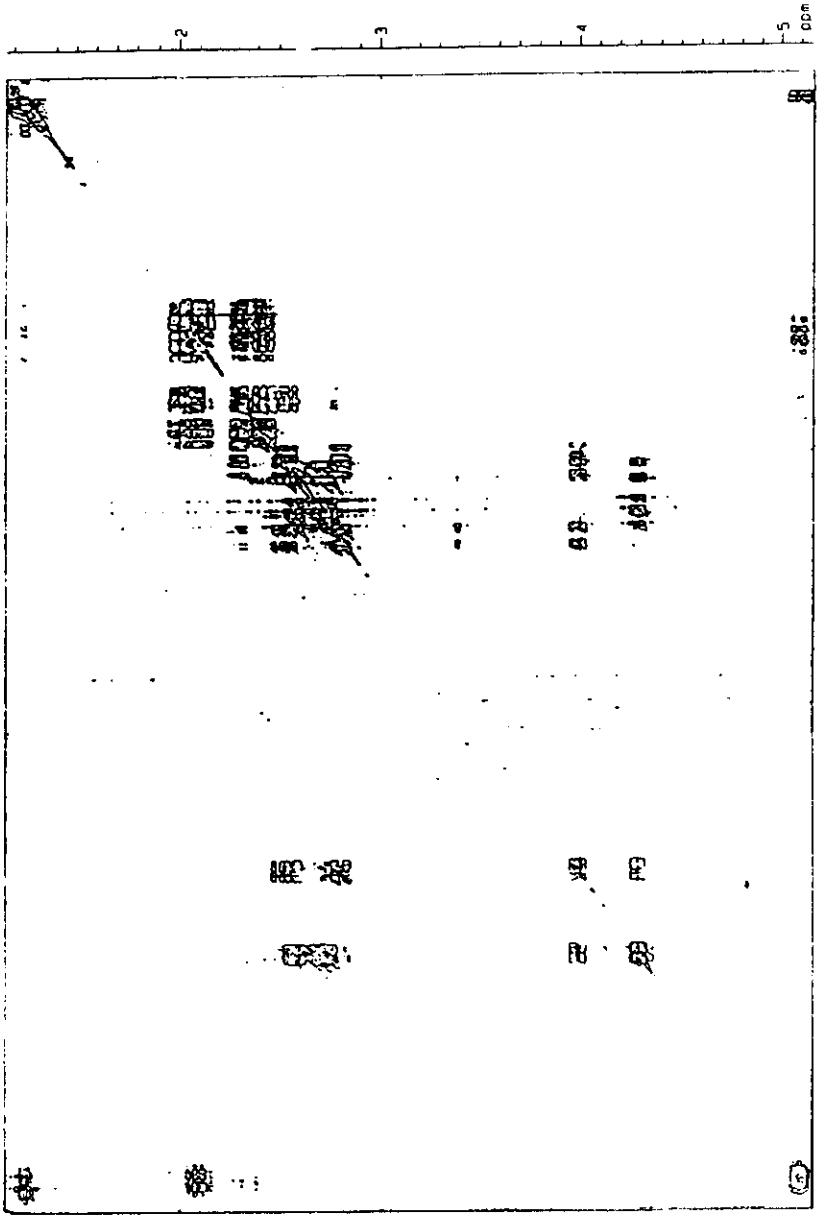


Figure 69 COSY (300 MHz) spectrum of VR-JOY13

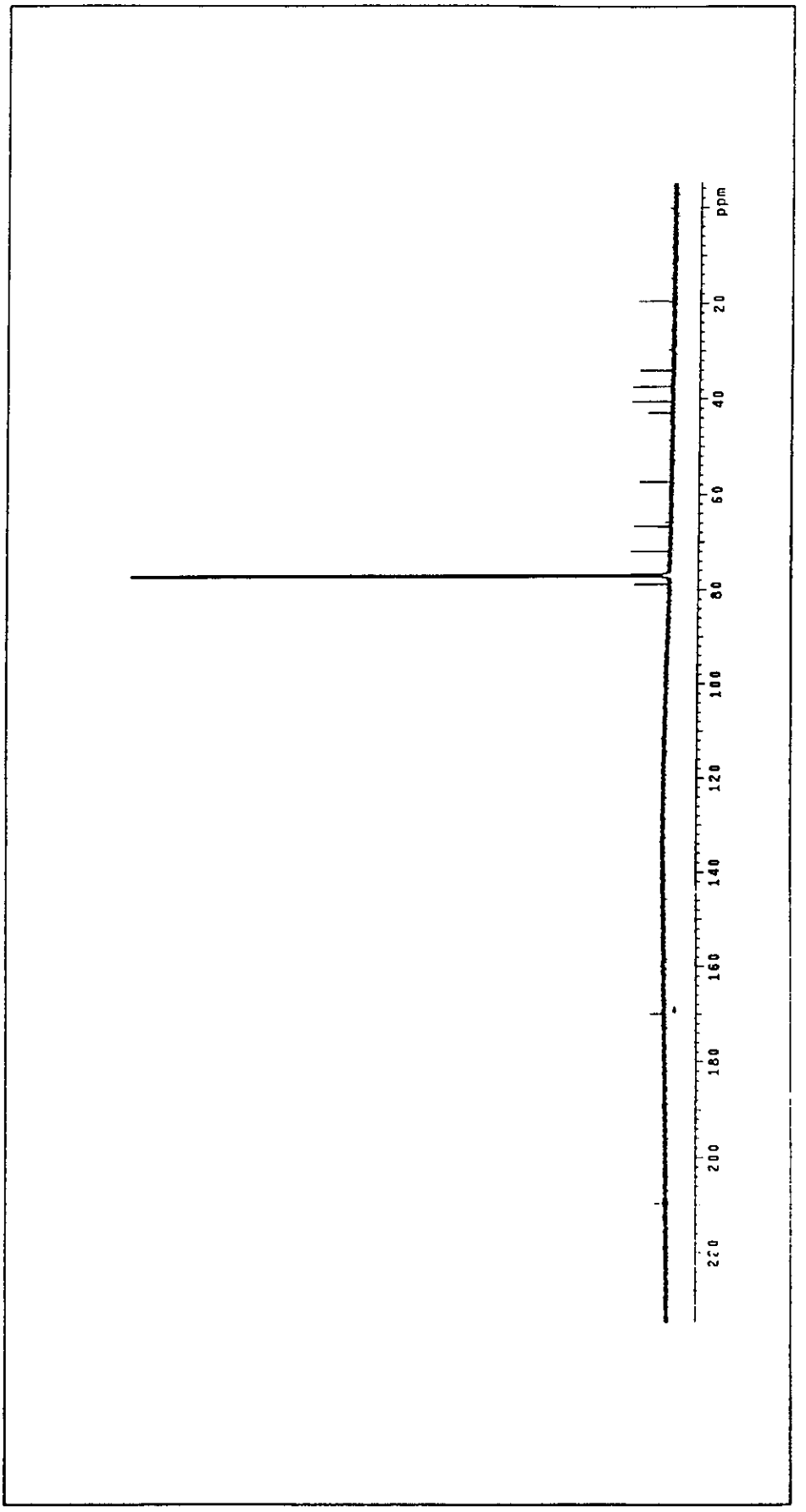


Figure 70 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of VR-JOY13

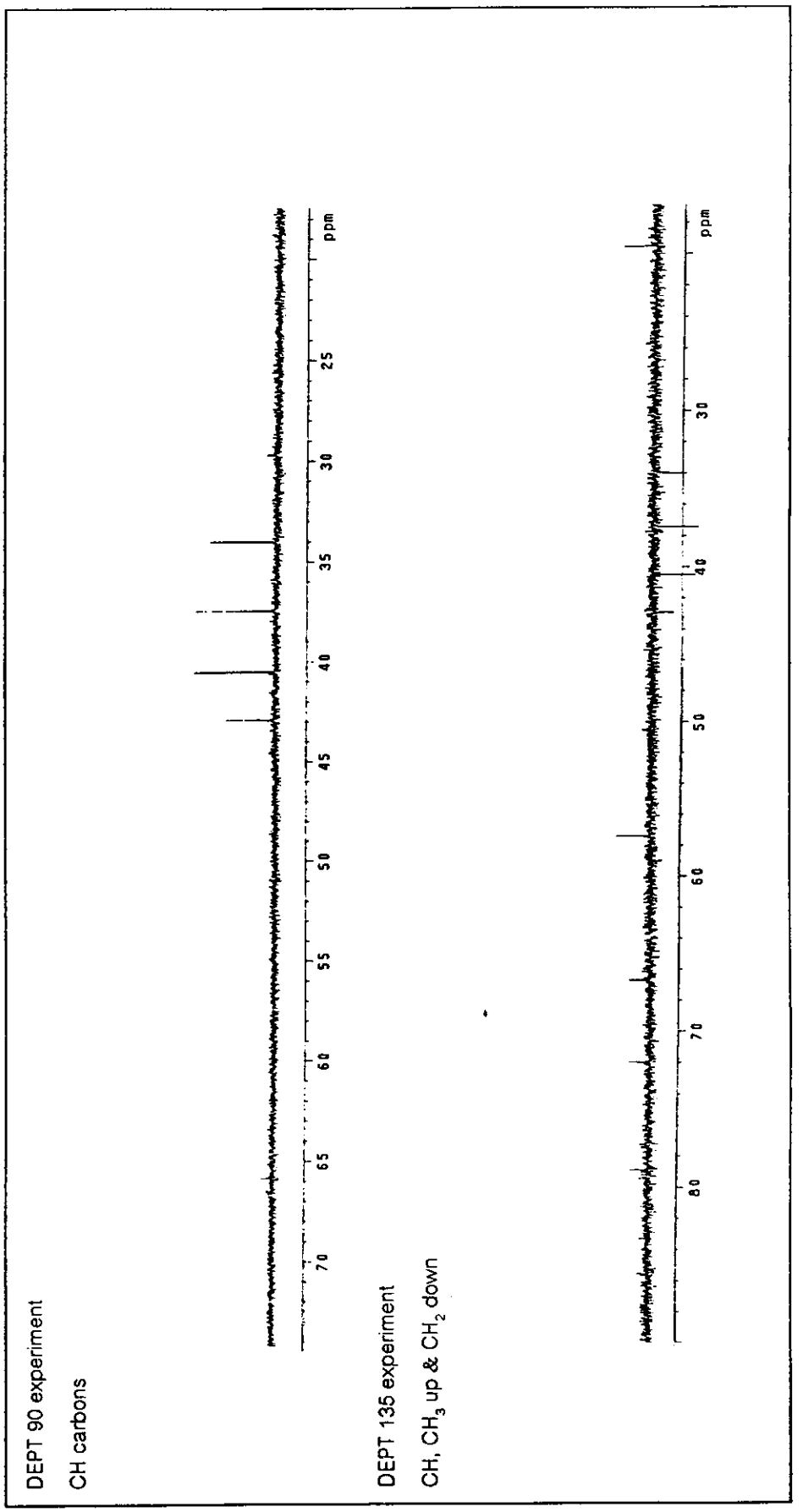


Figure 71 DEPT spectrum of VR-JOY13

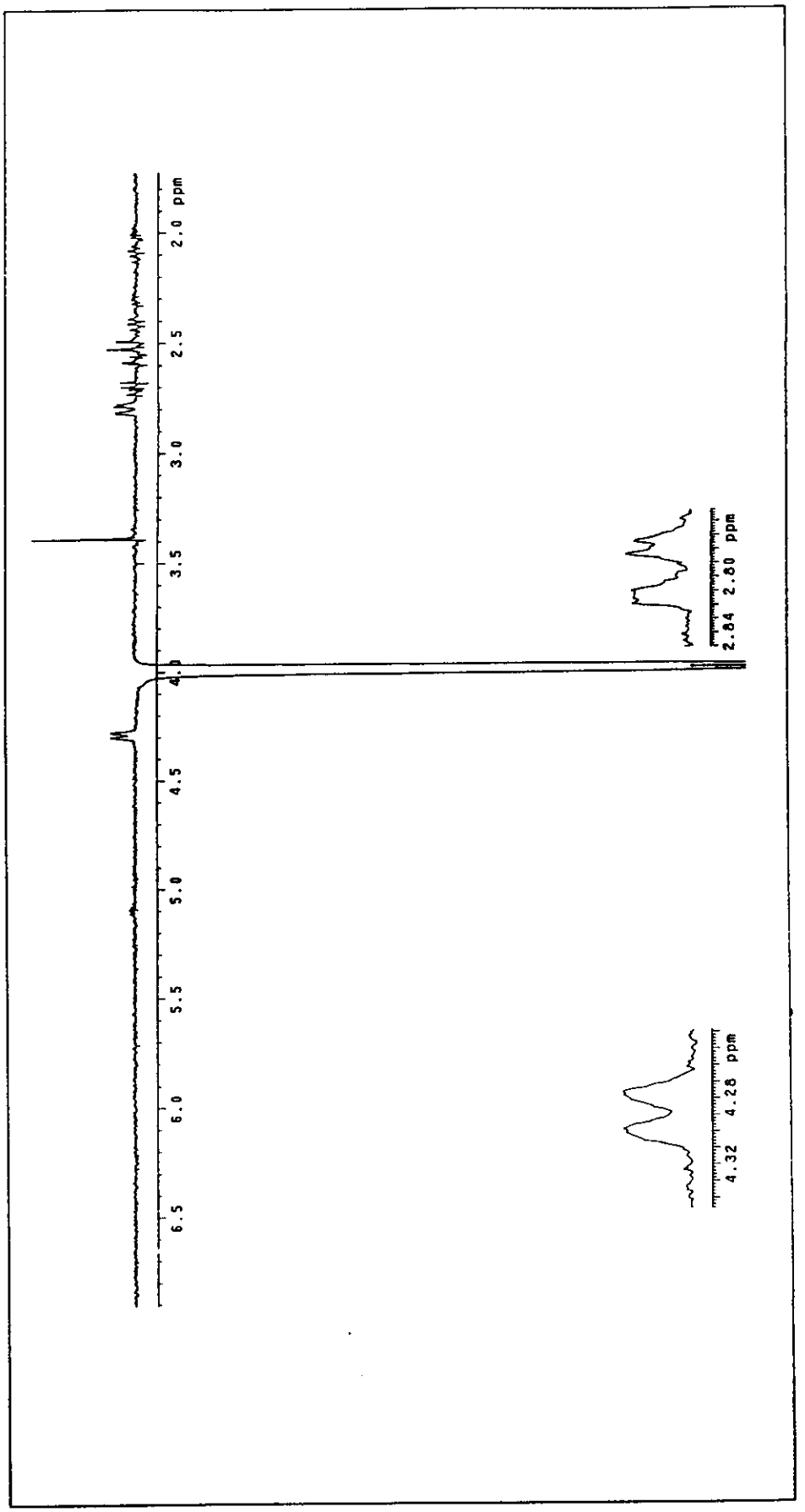


Figure 72 NOEDIFF spectrum of VR-JOY13 after irradiation at δ_H3.98

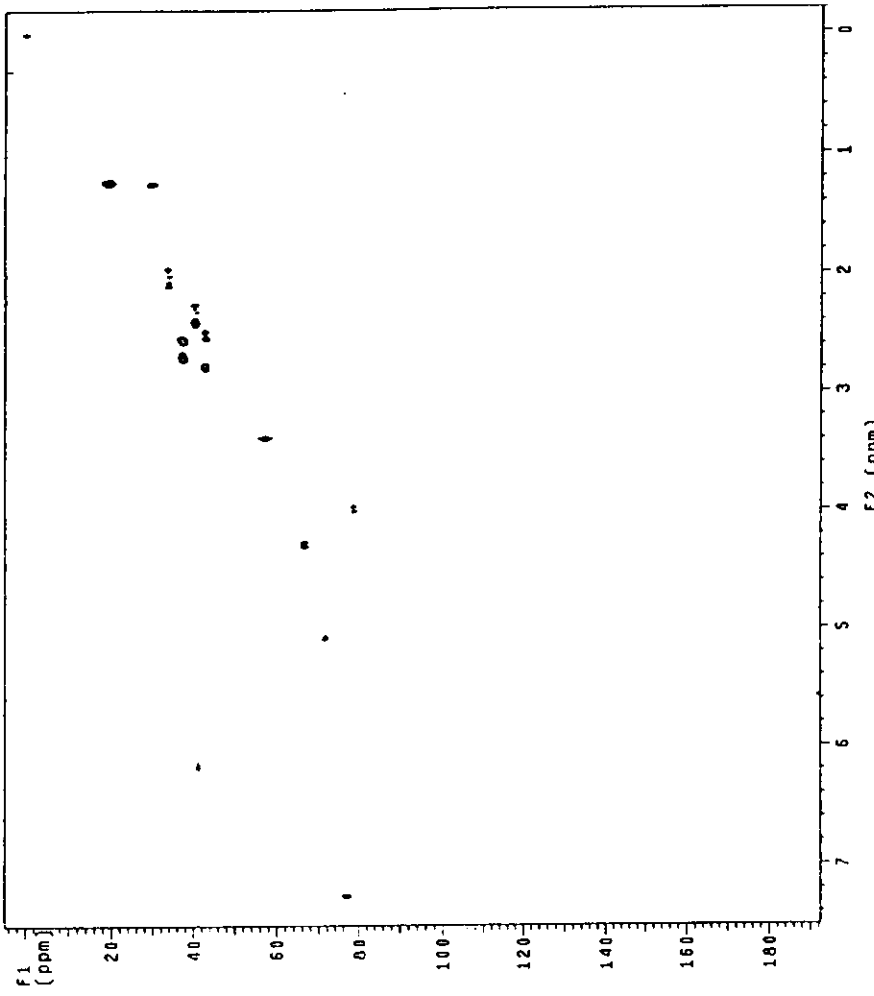


Figure 73 2D HMQC (500 MHz) spectrum of VR-JOY13

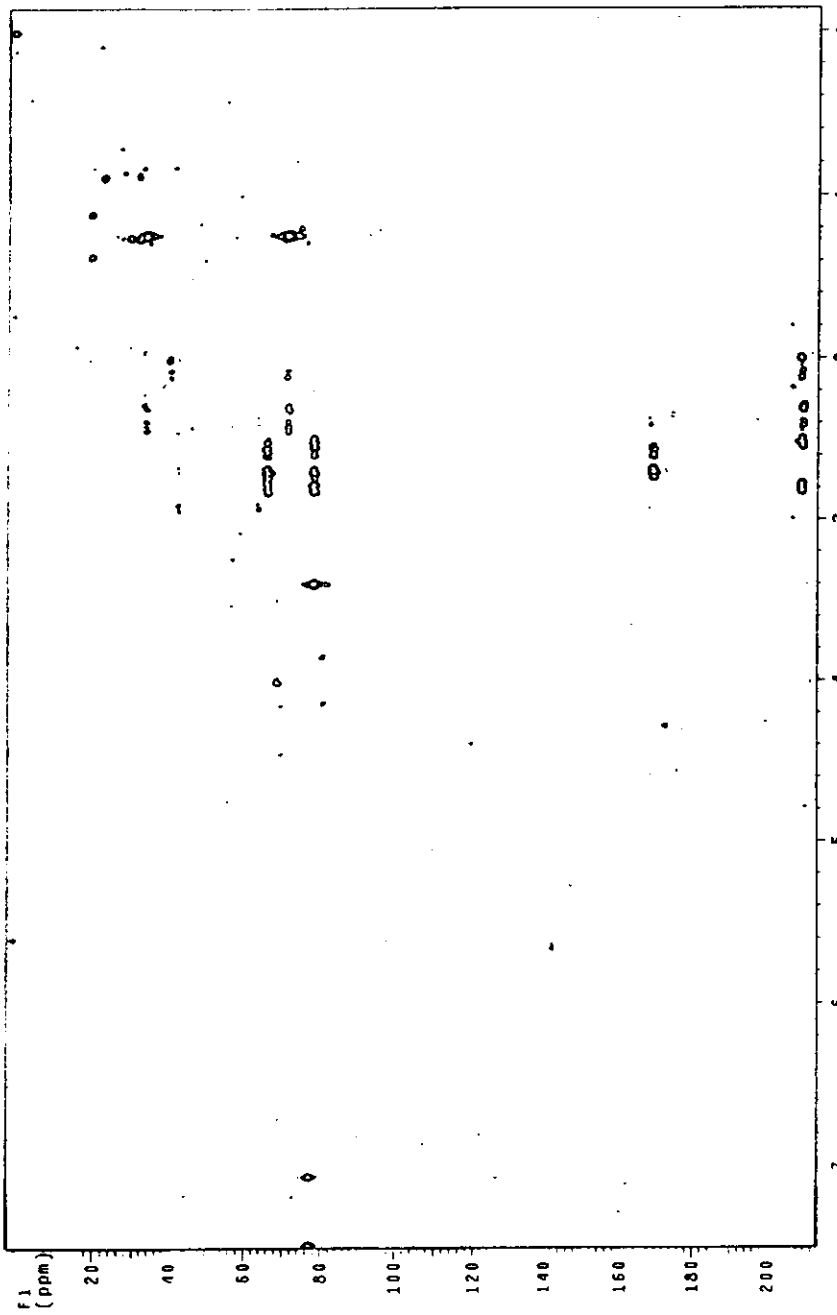


Figure 74 2D HMBC (500 MHz) spectrum of VR-JOY13

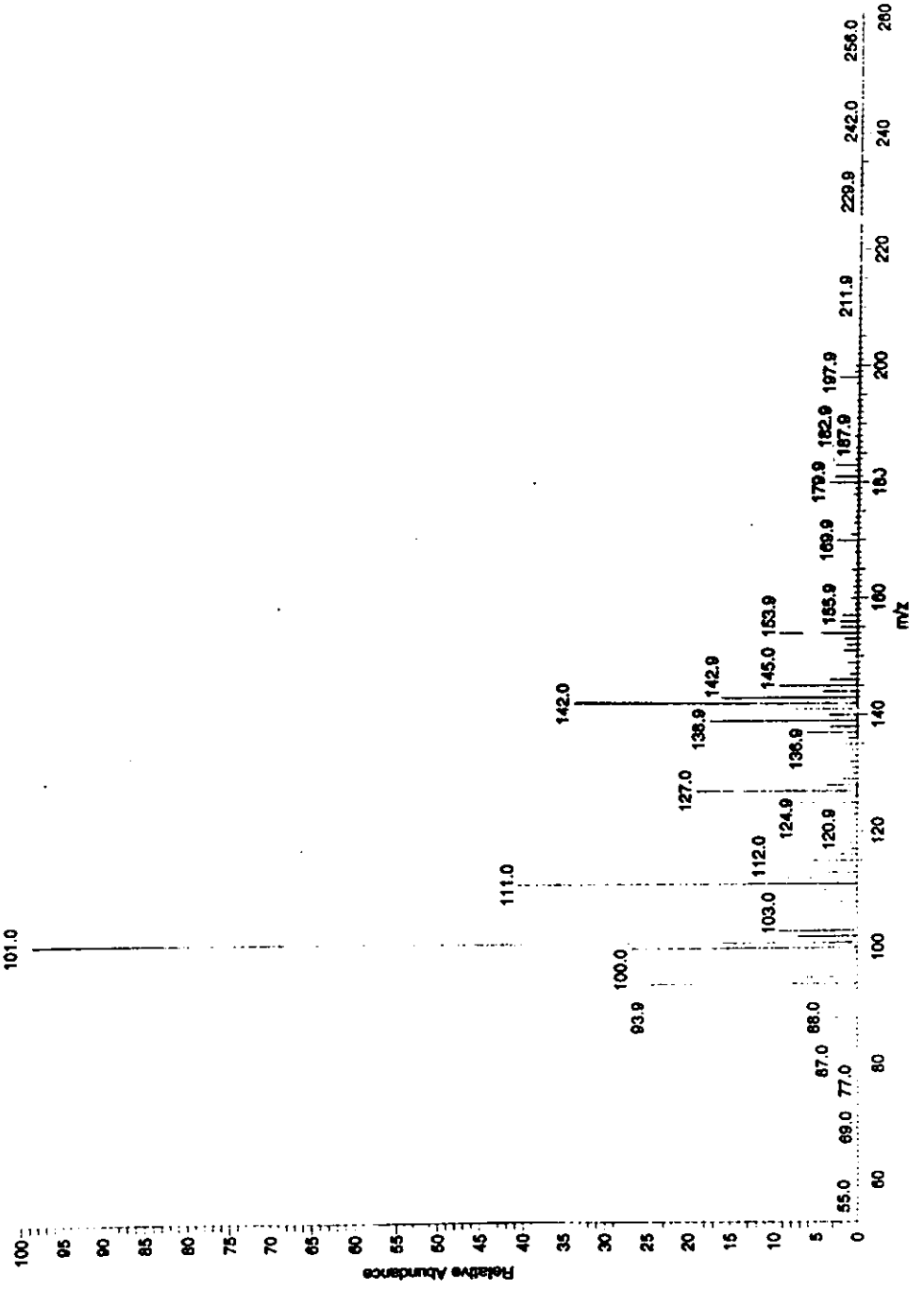


Figure 75 Mass spectrum of VR-JOY13

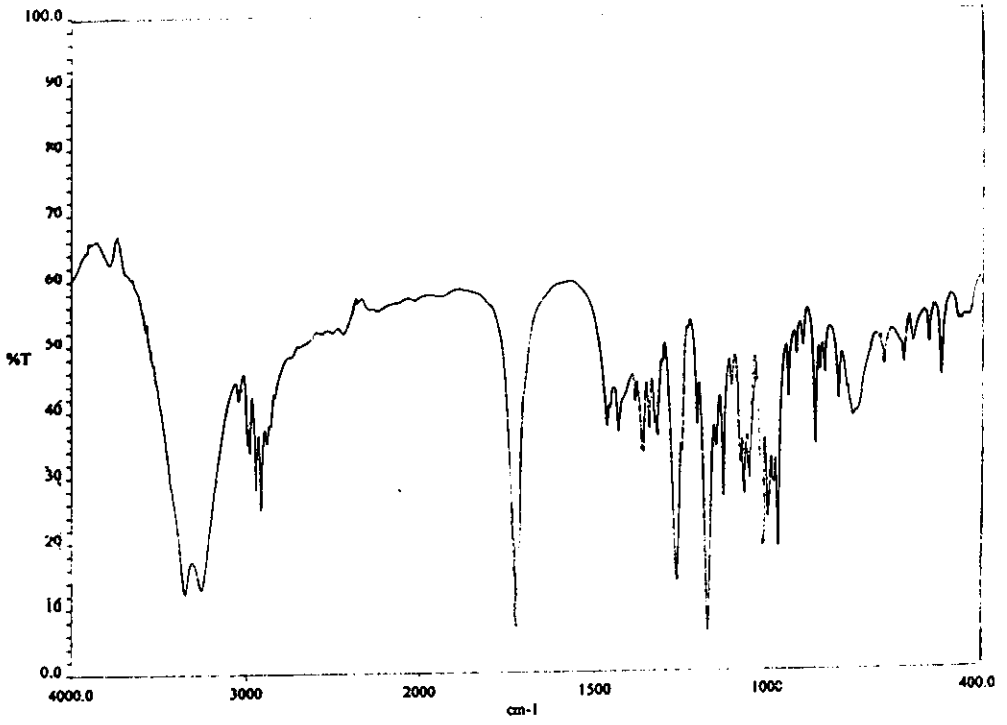


Figure 76 FT-IR (KBr) spectrum of VR-JOY11

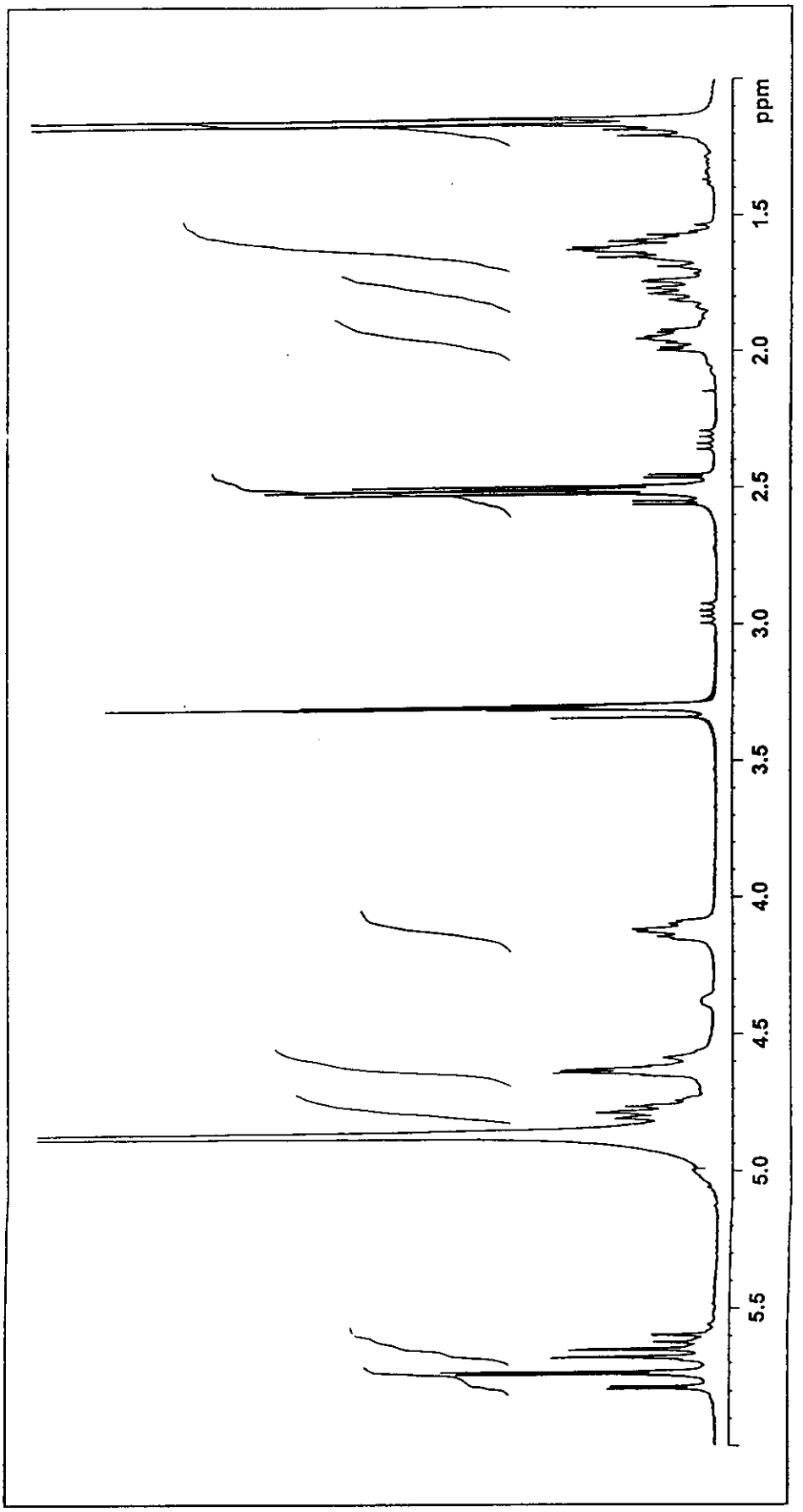


Figure 77 ^{1}H NMR (300 MHz) (CD_3OD) spectrum of VR-JOY11

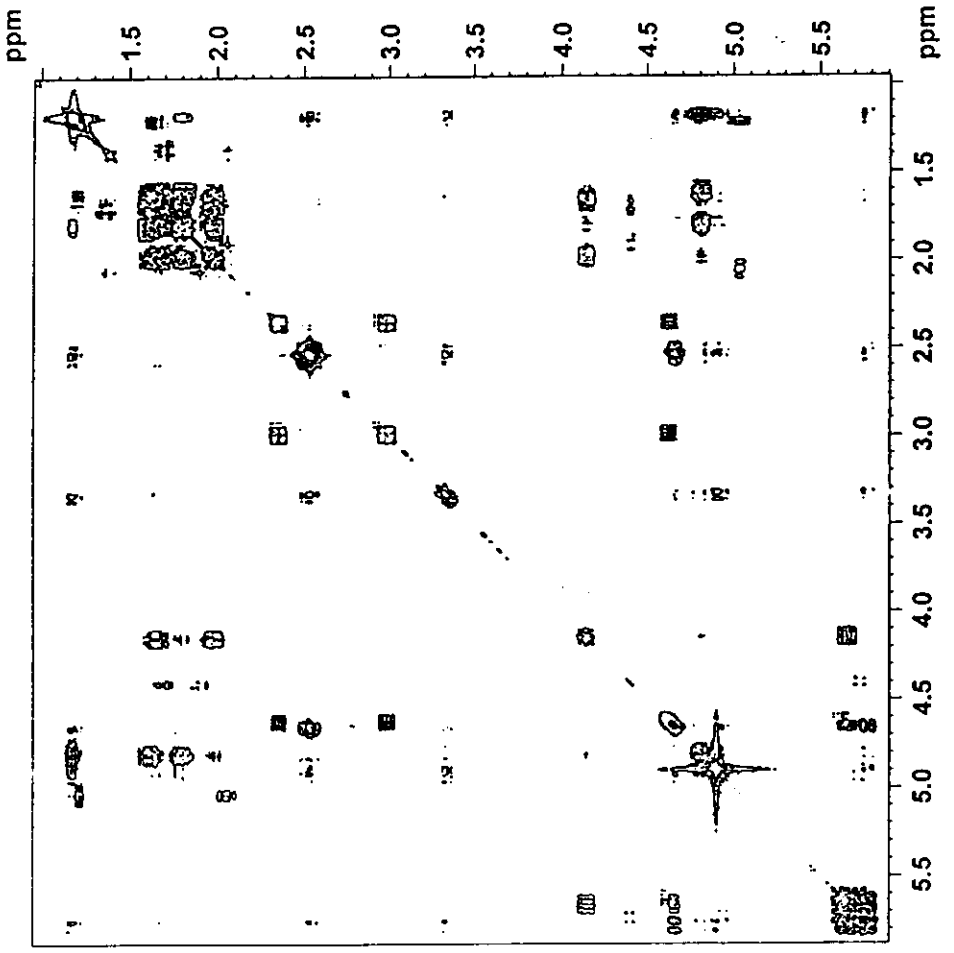


Figure 78 COSY (300 MHz) spectrum of VR-JOY11

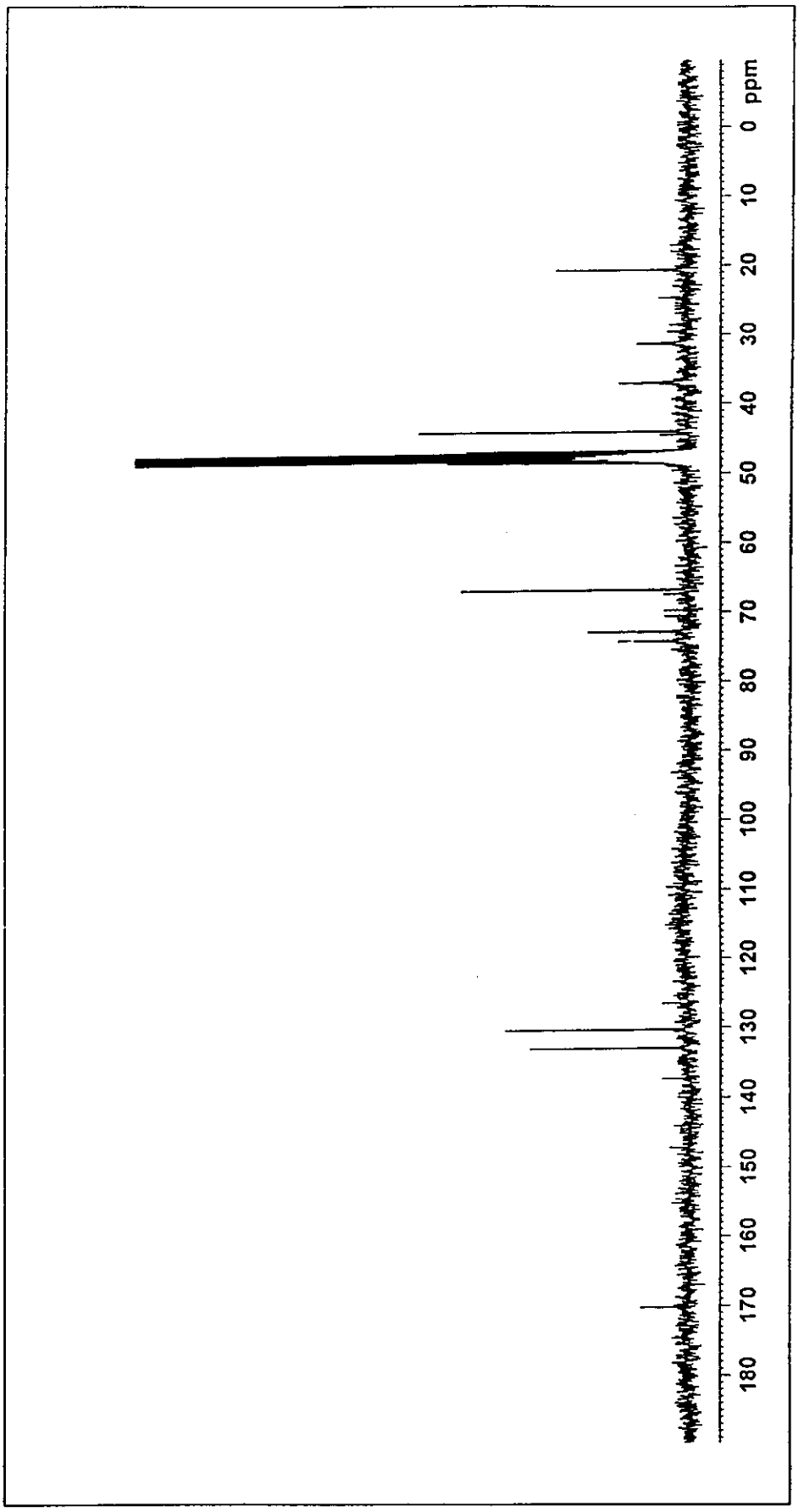


Figure 79 ^{13}C NMR (75 MHz) (CD_3OD) spectrum of VR-JOY11

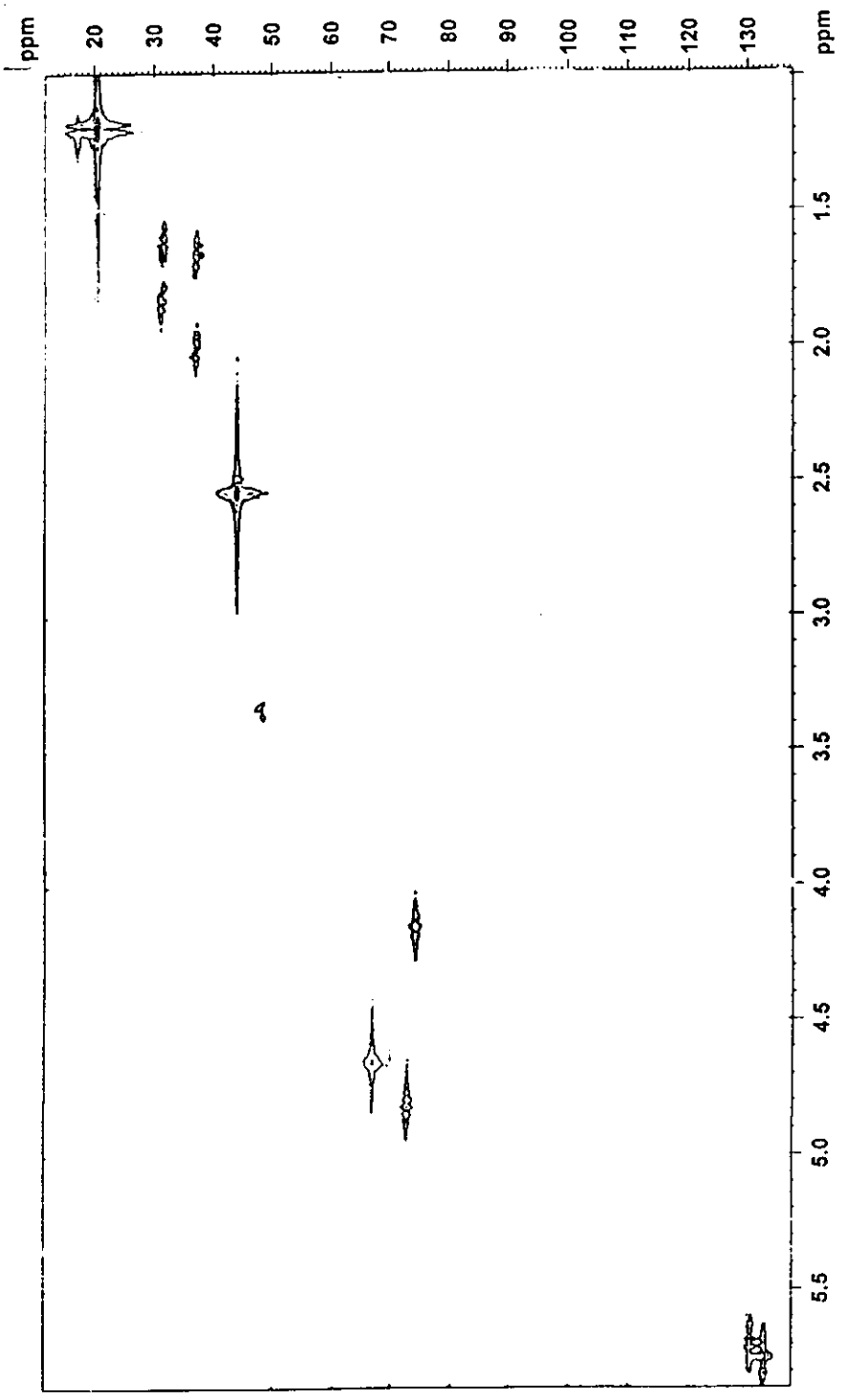


Figure 80 2D HMQC (300 MHz) spectrum of VR-JOY11

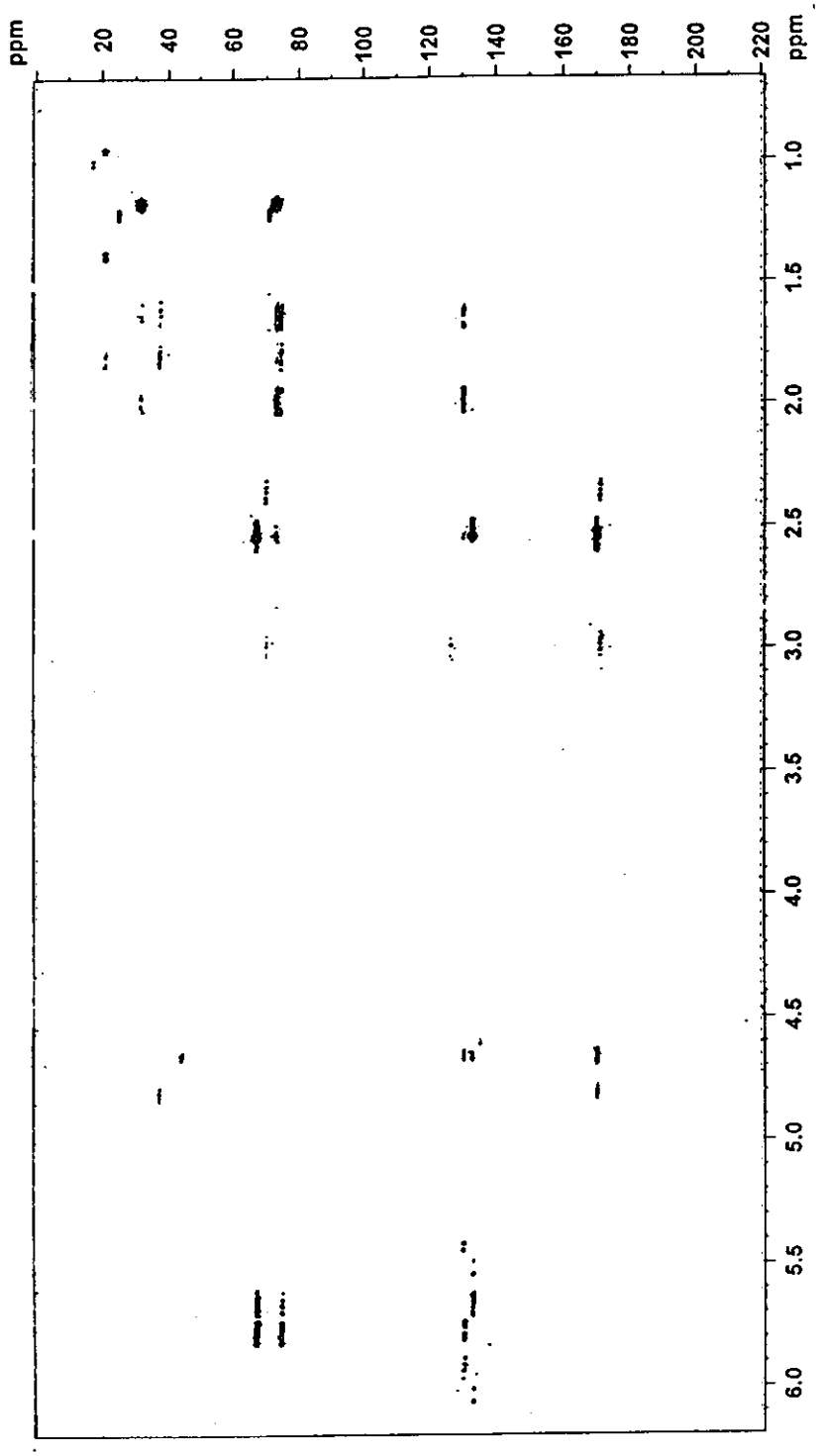


Figure 81 2D HMBC (300 MHz) spectrum of VR-JOY11

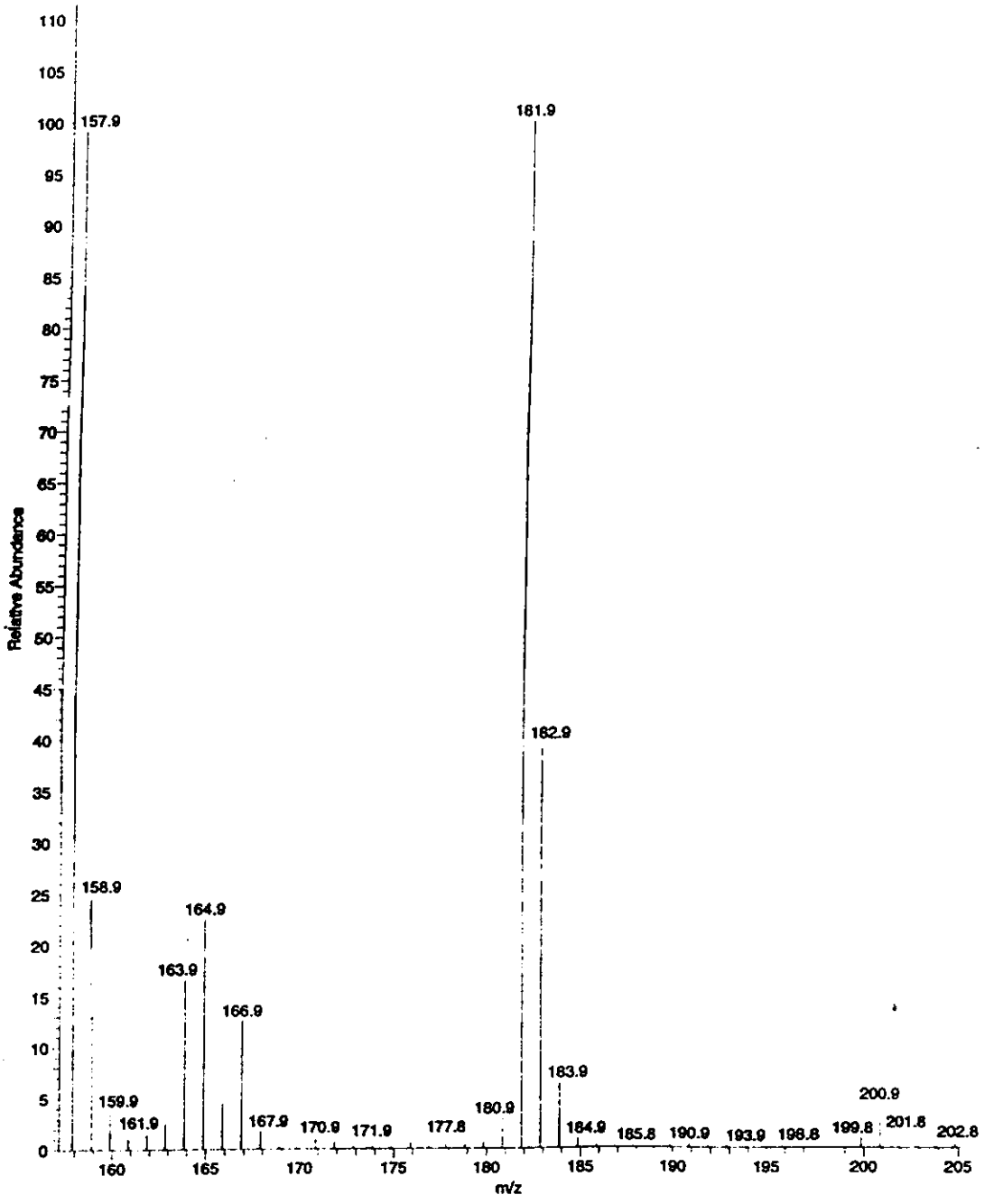


Figure 82 Mass spectrum of VR-JOY11

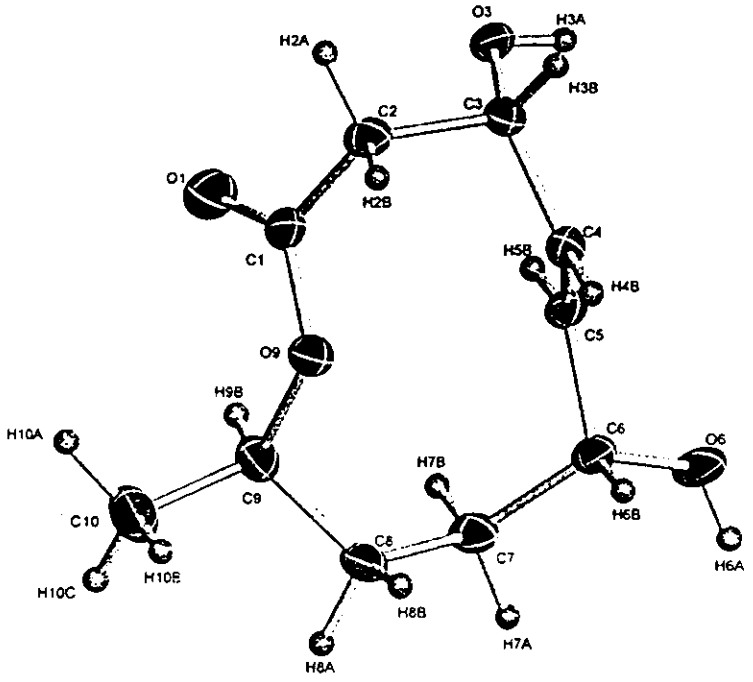


Figure 83 X-ray structure of VR-JOY11

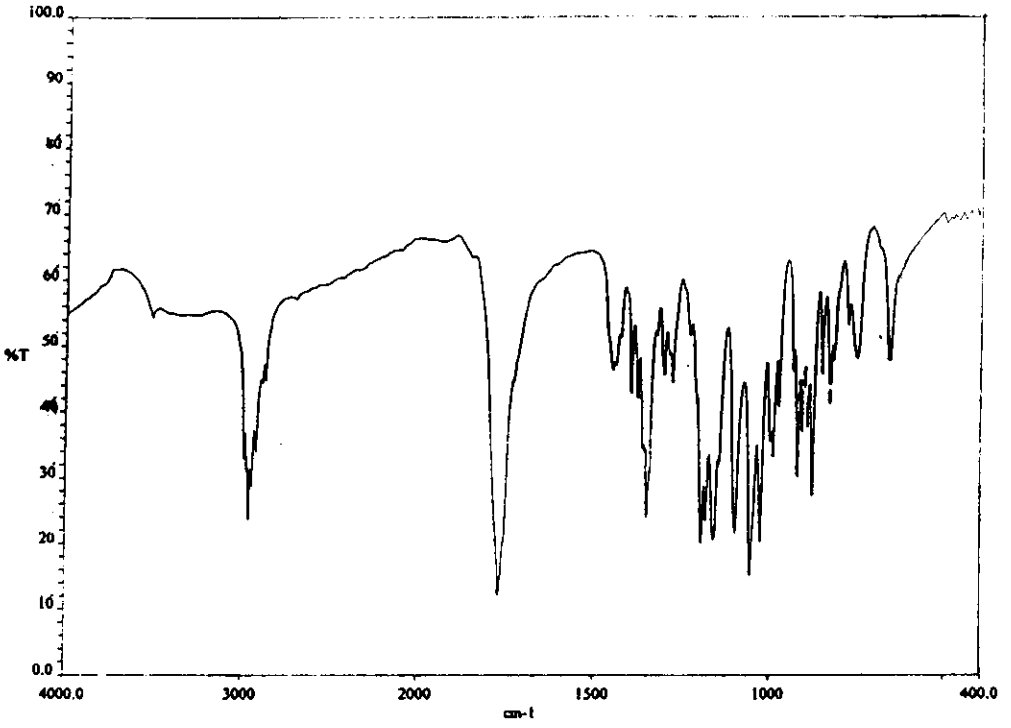


Figure 84 FT-IR (KBr) spectrum of VR-JOY7

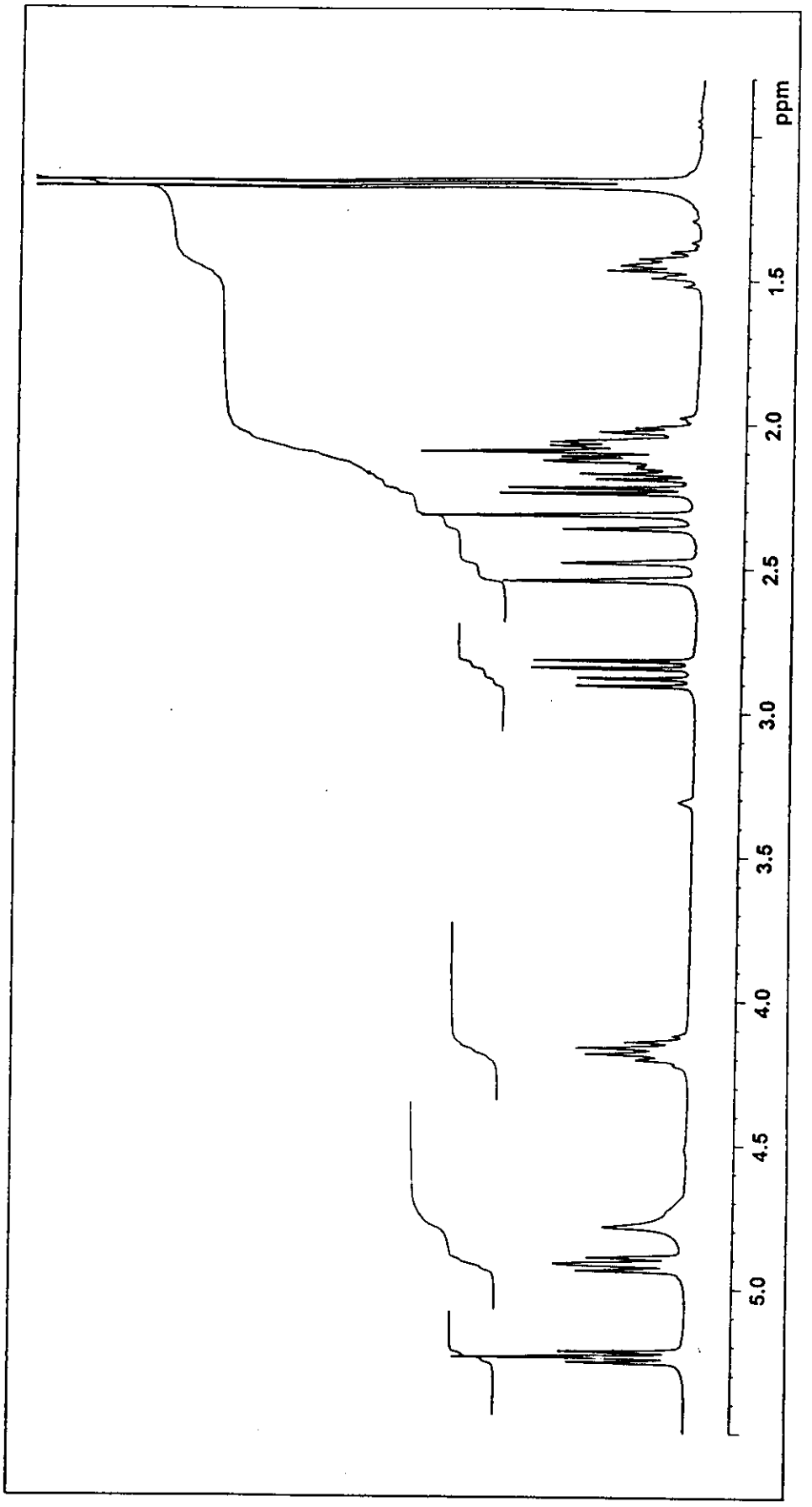


Figure 85 ^{1}H NMR (300 MHz) (CD_3OD) spectrum of VR-JOY7

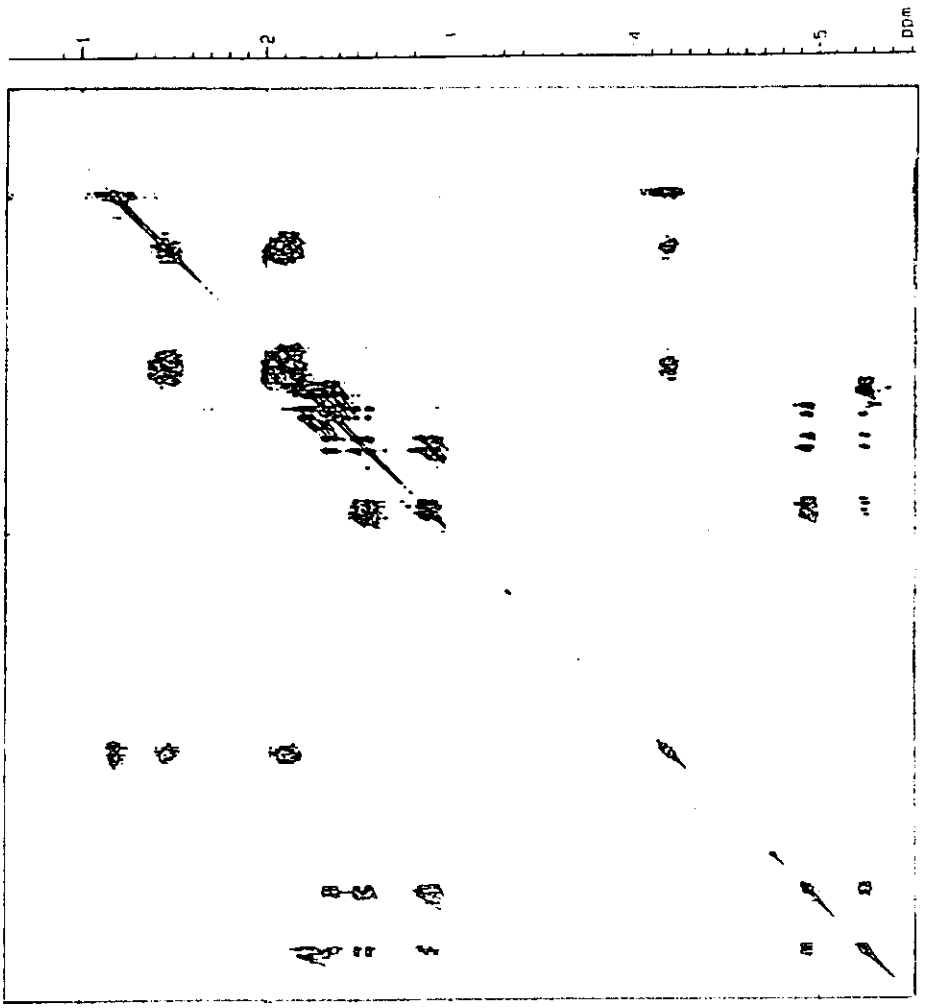


Figure 86 COSY (300 MHz) spectrum of VR-JOY7

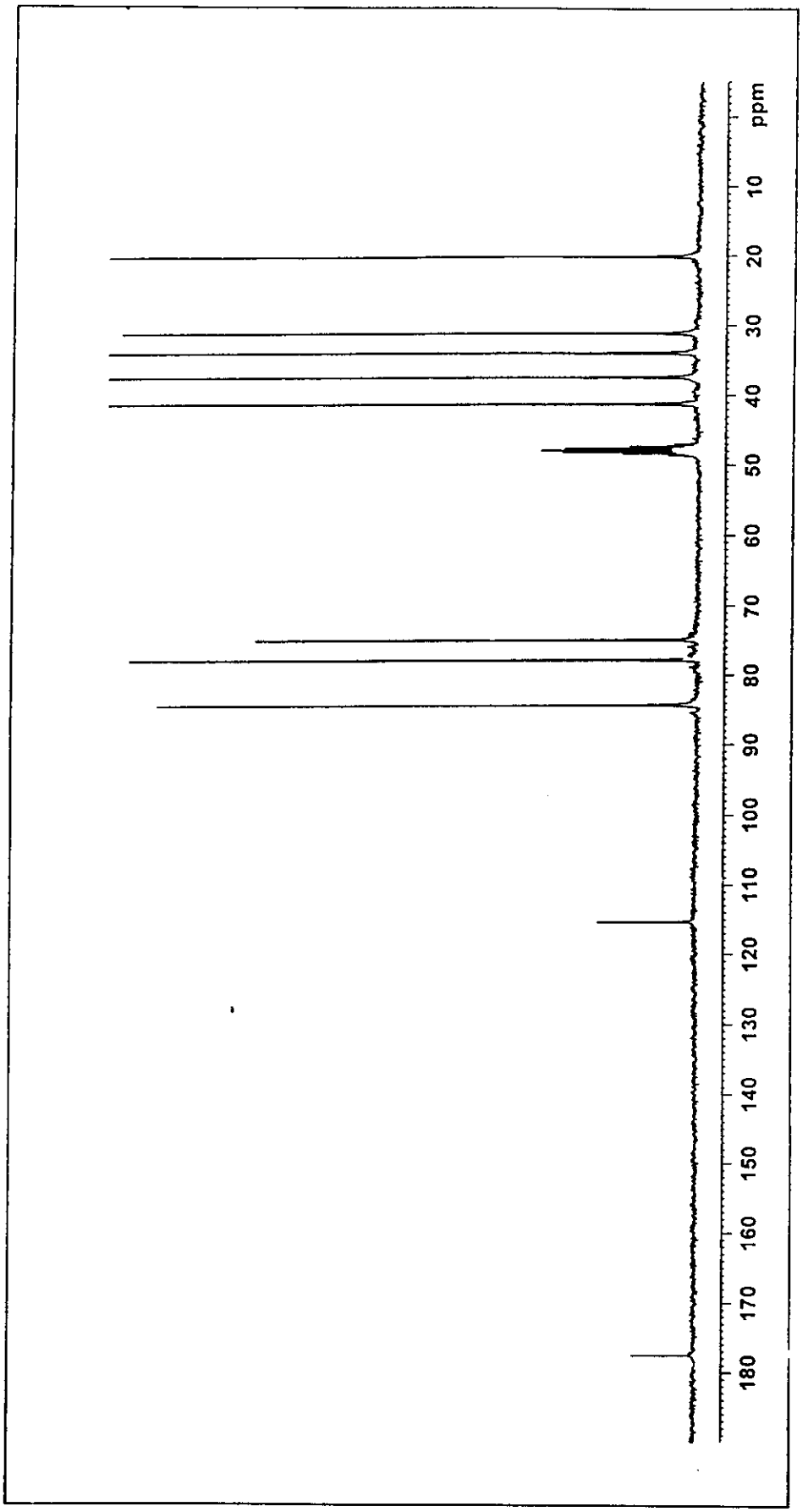


Figure 87 ^{13}C NMR (75 MHz) (CD_3OD) spectrum of VR-JOY7

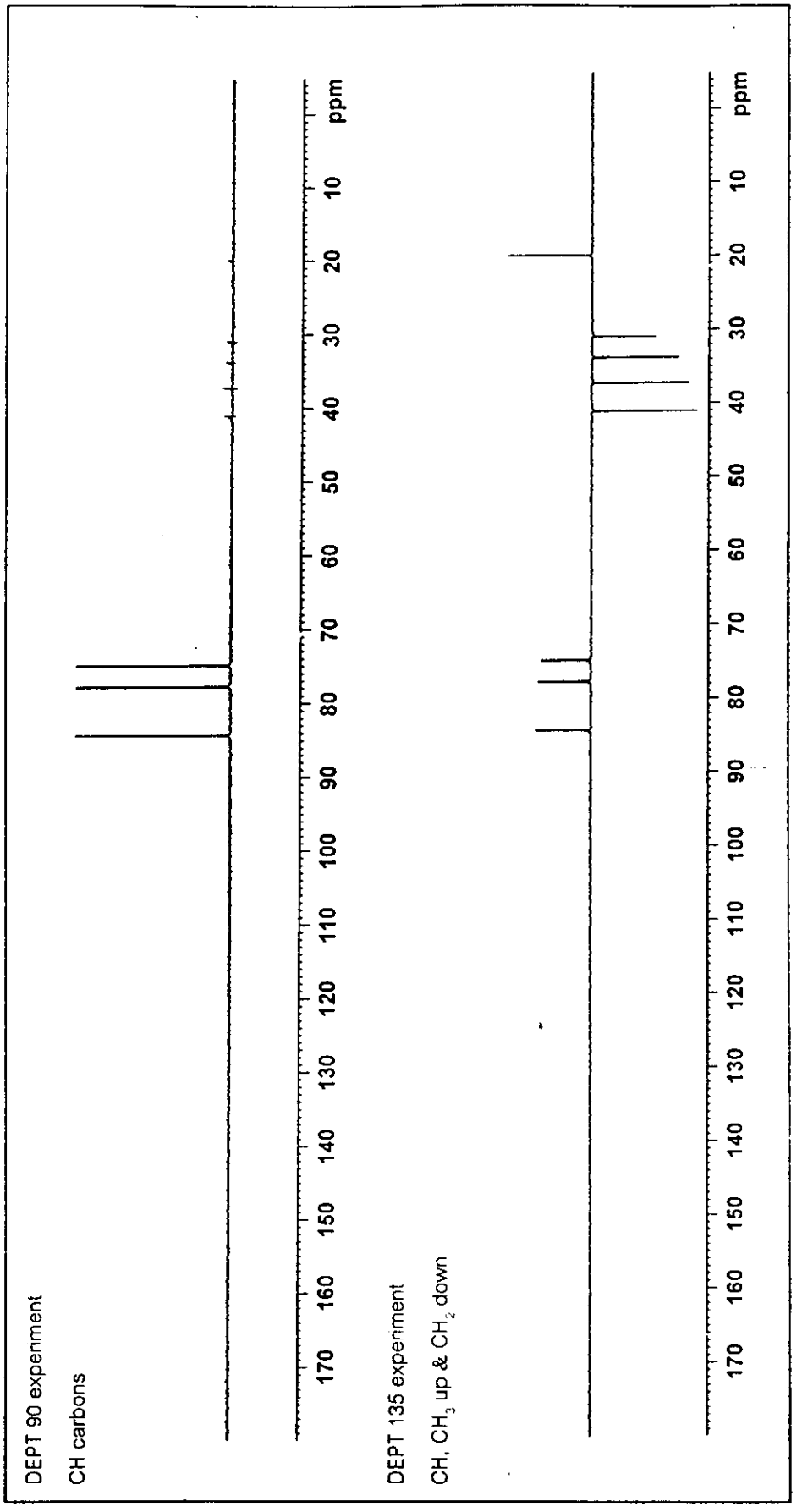


Figure 88 DEPT spectrum of VR-JOY7

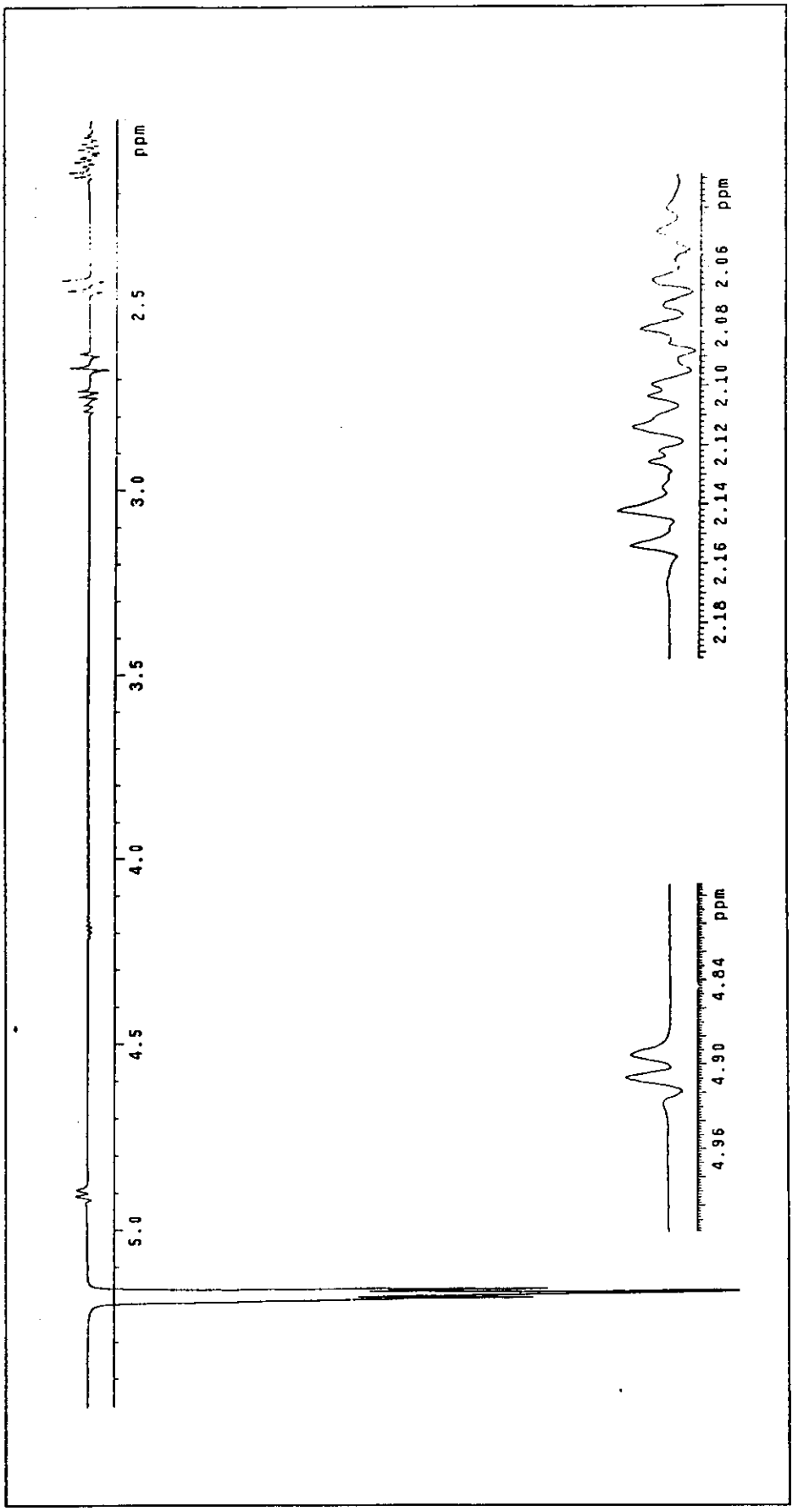


Figure 89 NOEDIFF spectrum of VR-JOY7 after irradiation at δ_H5.24

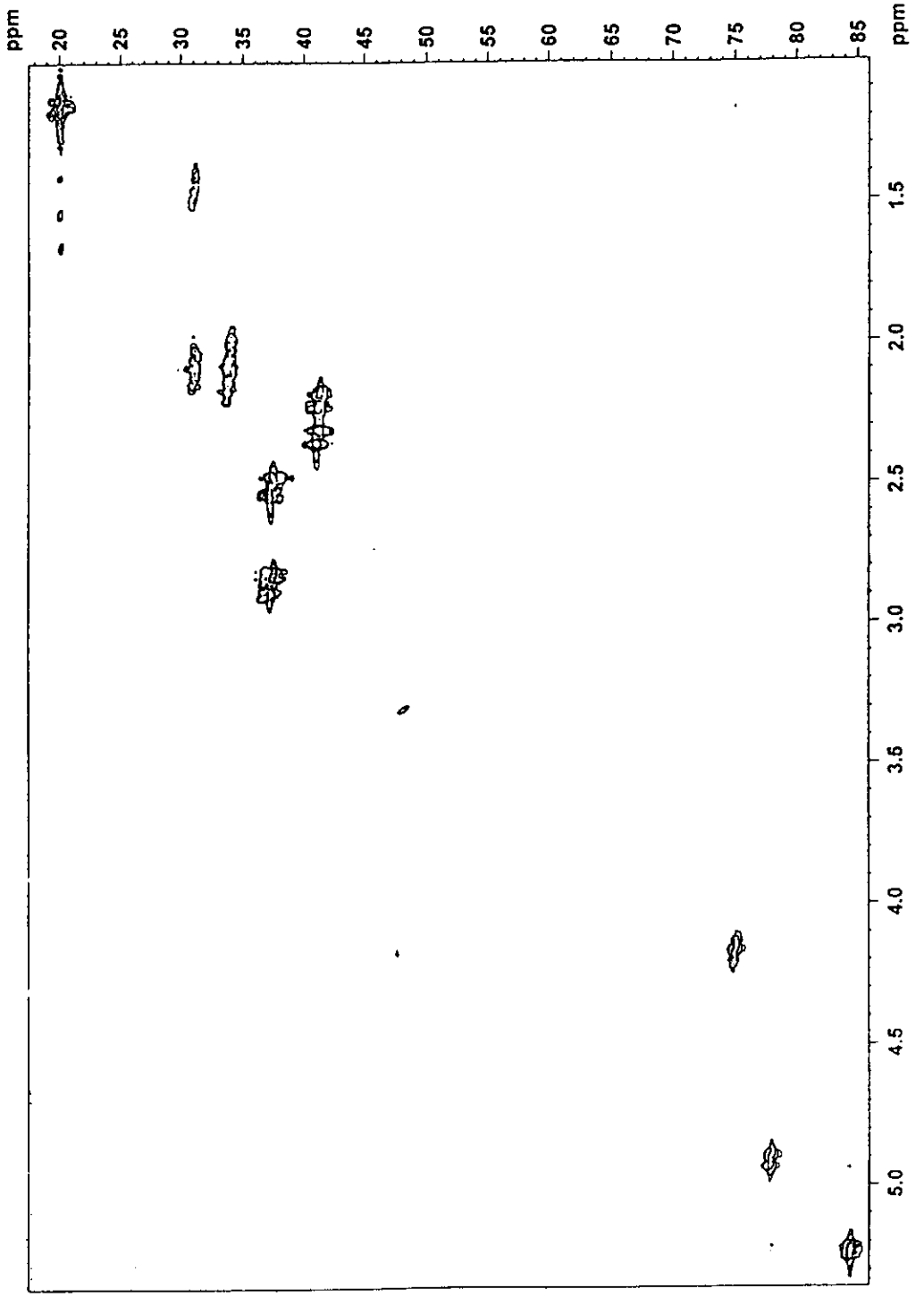


Figure 90 2D HMQC (300 MHz) spectrum of VR-JOY7

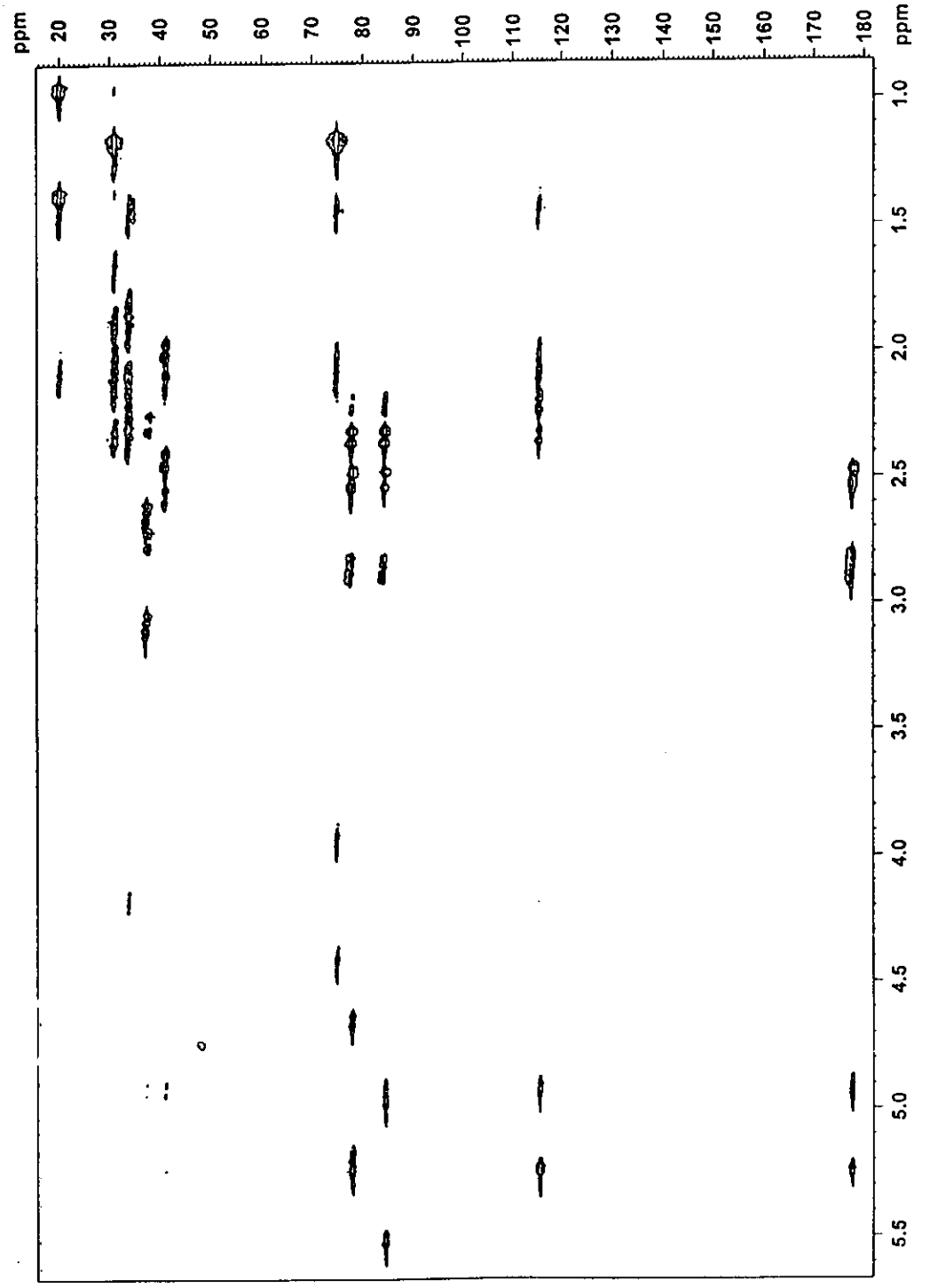


Figure 91 2D HMBC (300 MHz) spectrum of VR-JOY7

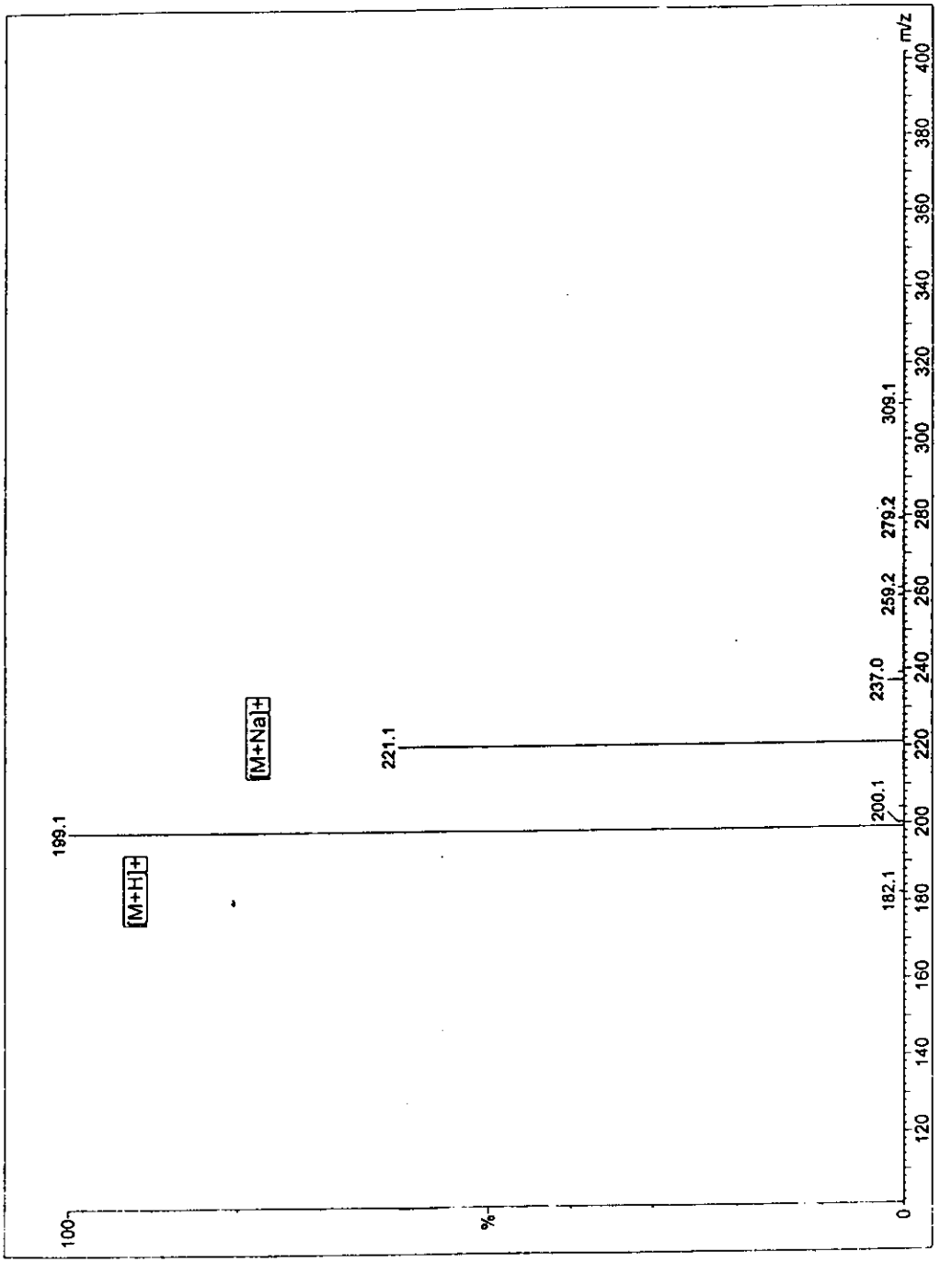


Figure 92 Mass spectrum of VR-JOY7

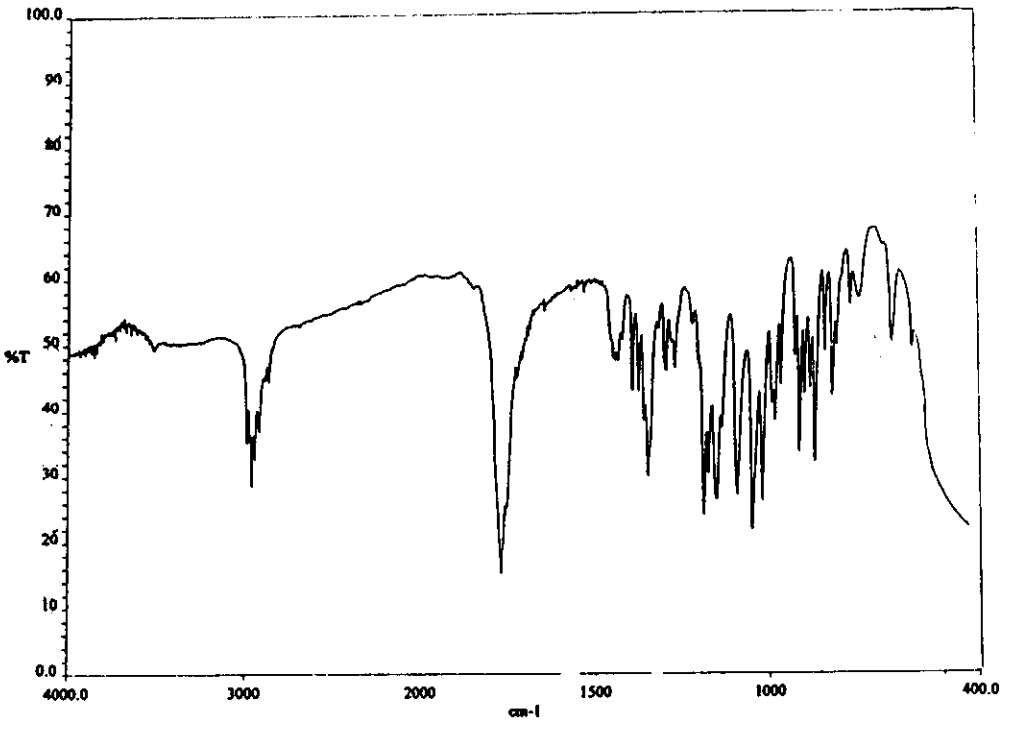


Figure 93 FT-IR (neat) spectrum of VR-JOY8

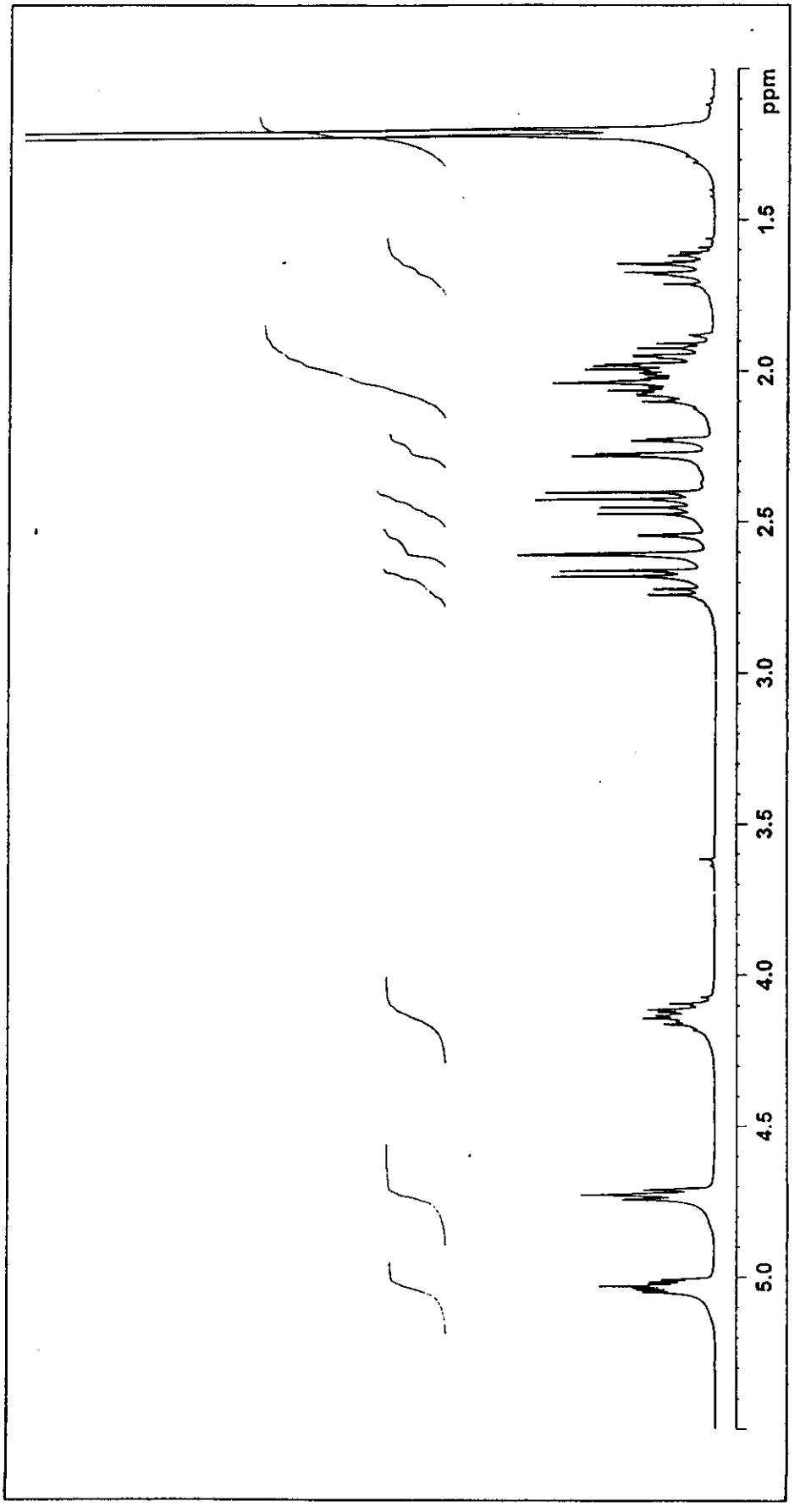


Figure 94 ^{1}H NMR (300 MHz) ($CDCl_3$+CD_3OD) spectrum of VR-JOY8

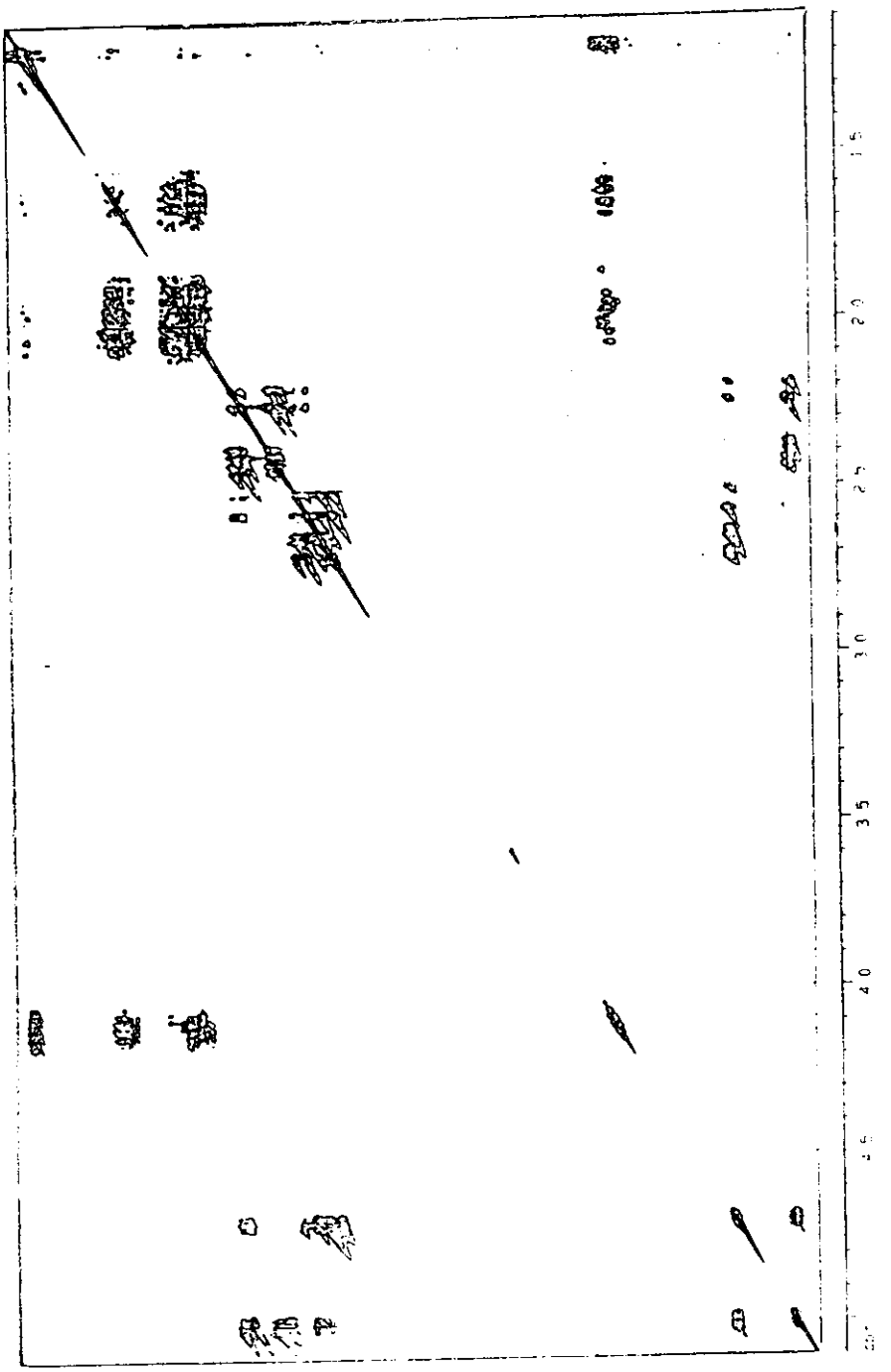


Figure 95 COSY (300 MHz) spectrum of VR-JOY8

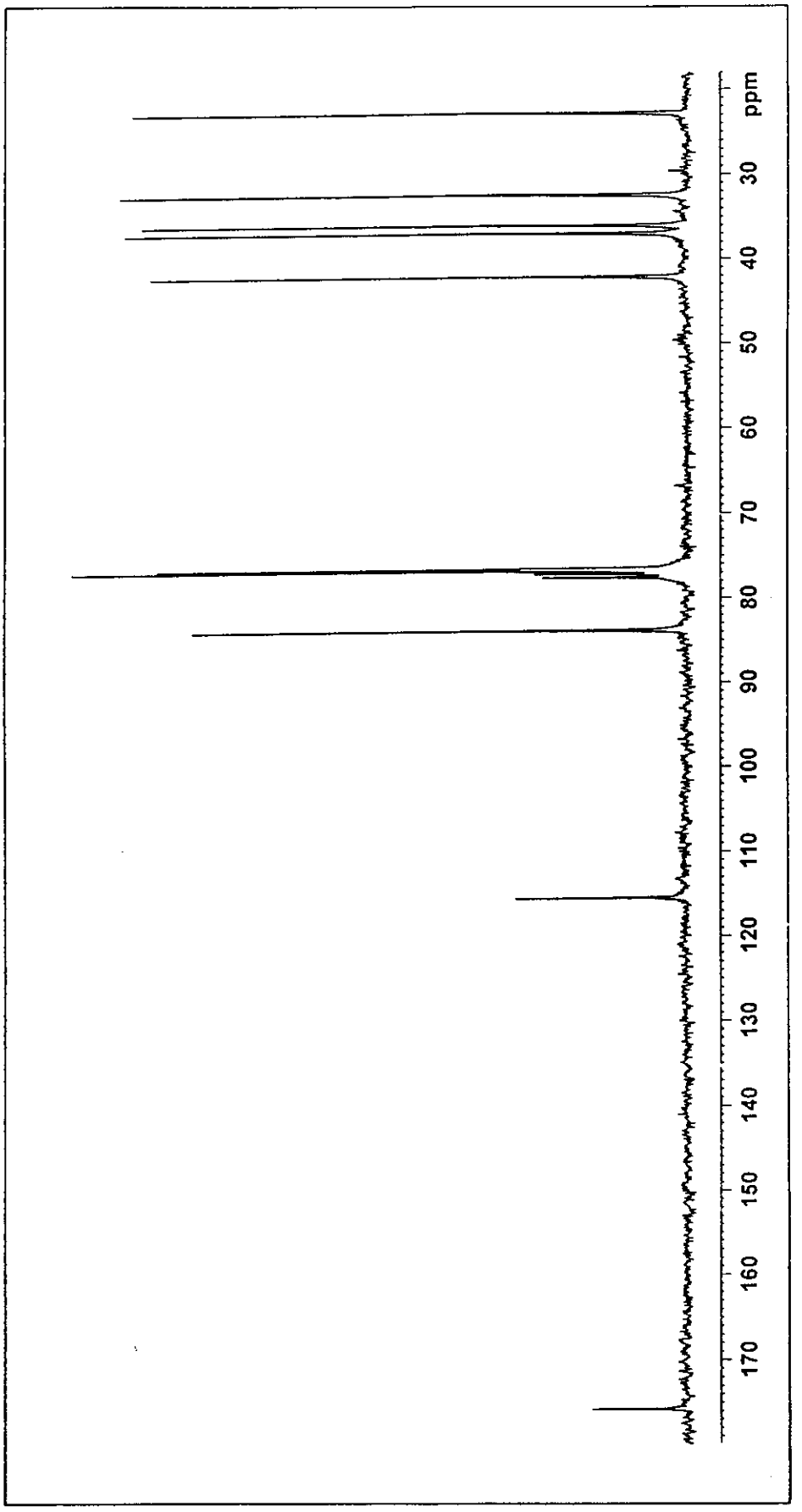


Figure 96 ^{13}C NMR (75 MHz) ($CDCl_3$+CD_3OD) spectrum of VR-JOY8

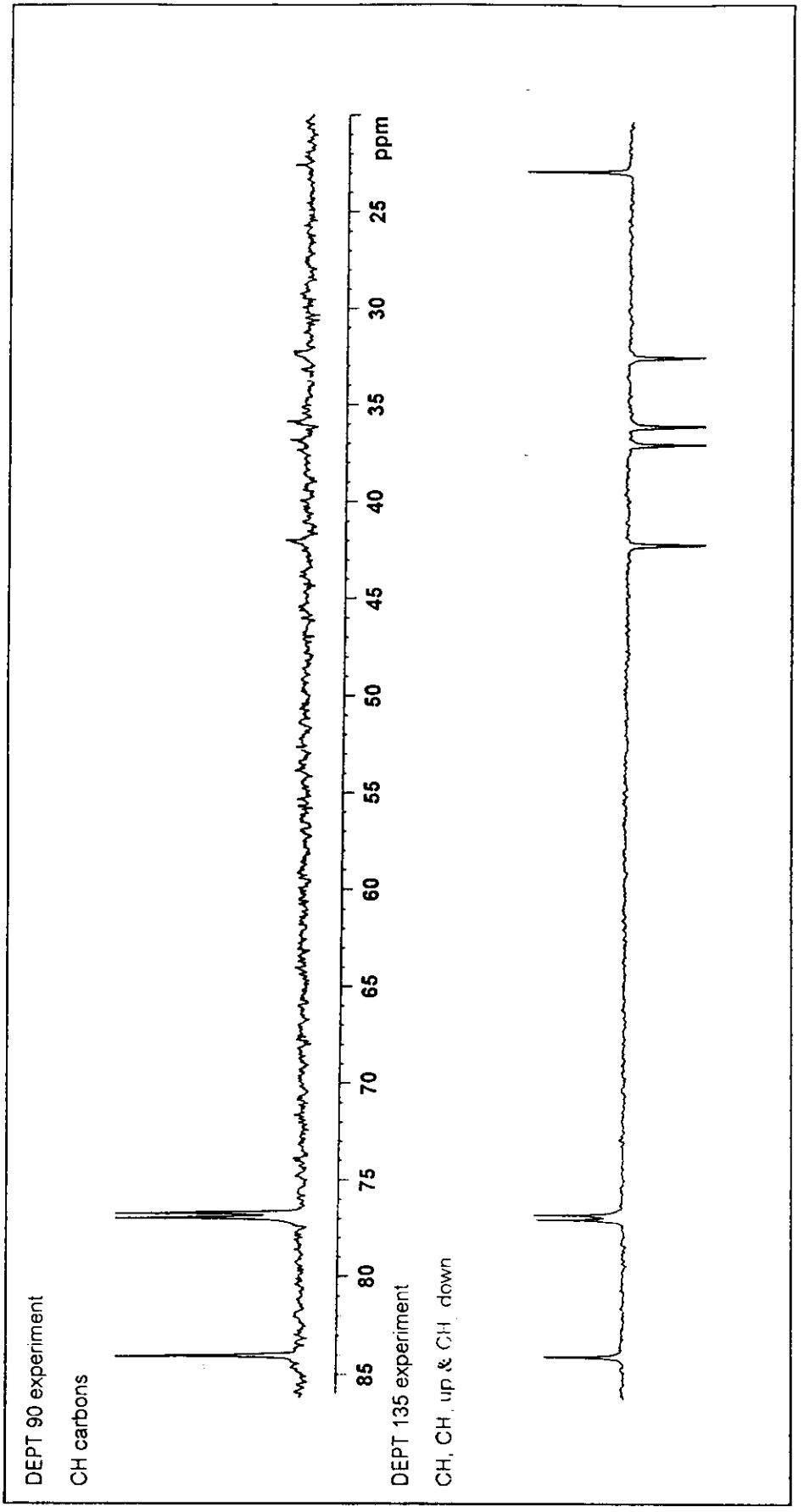


Figure 97 DEPT spectrum of VR-JOY8

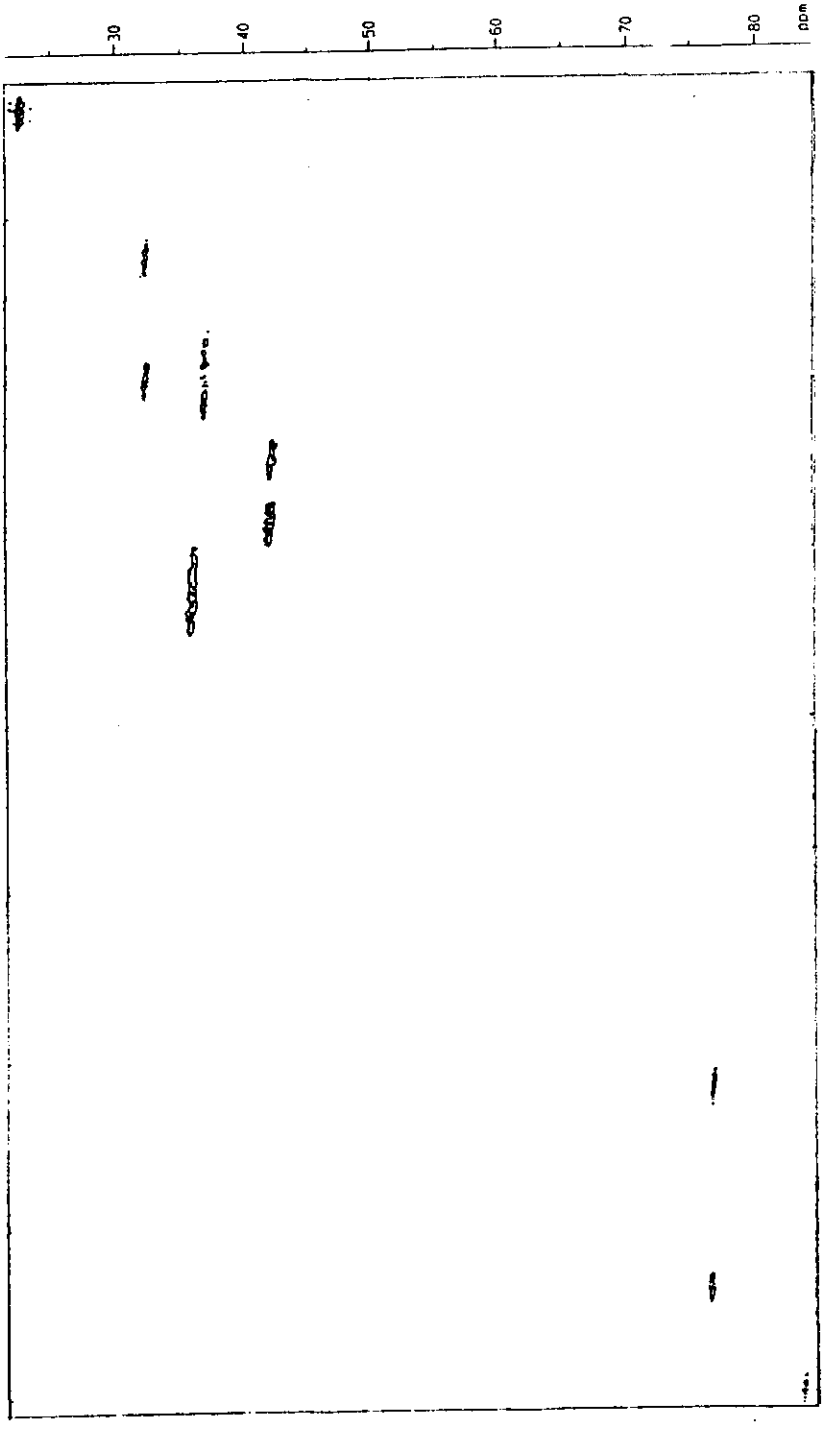


Figure 98 2D HMQC (300 MHz) spectrum of VR-JOY8

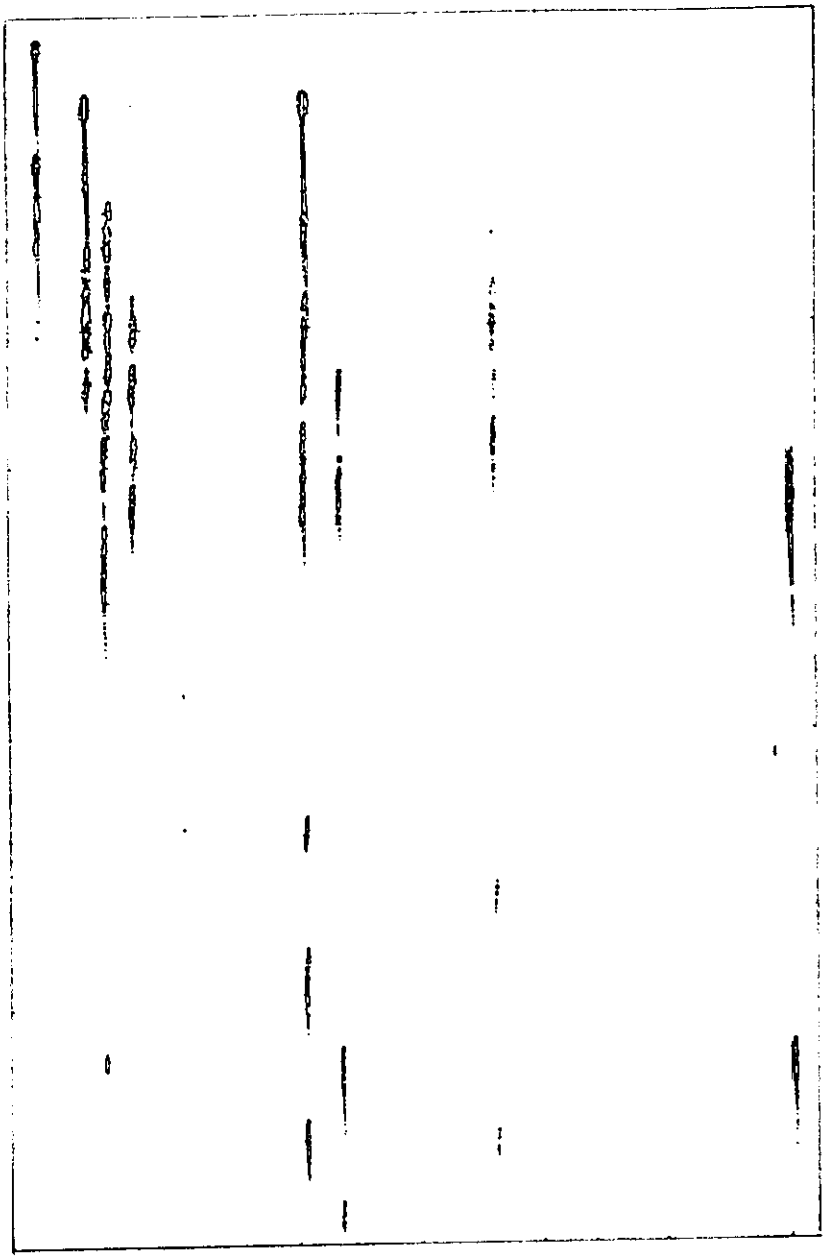


Figure 99 2D HMBC (300 MHz) spectrum of VR-JOY8

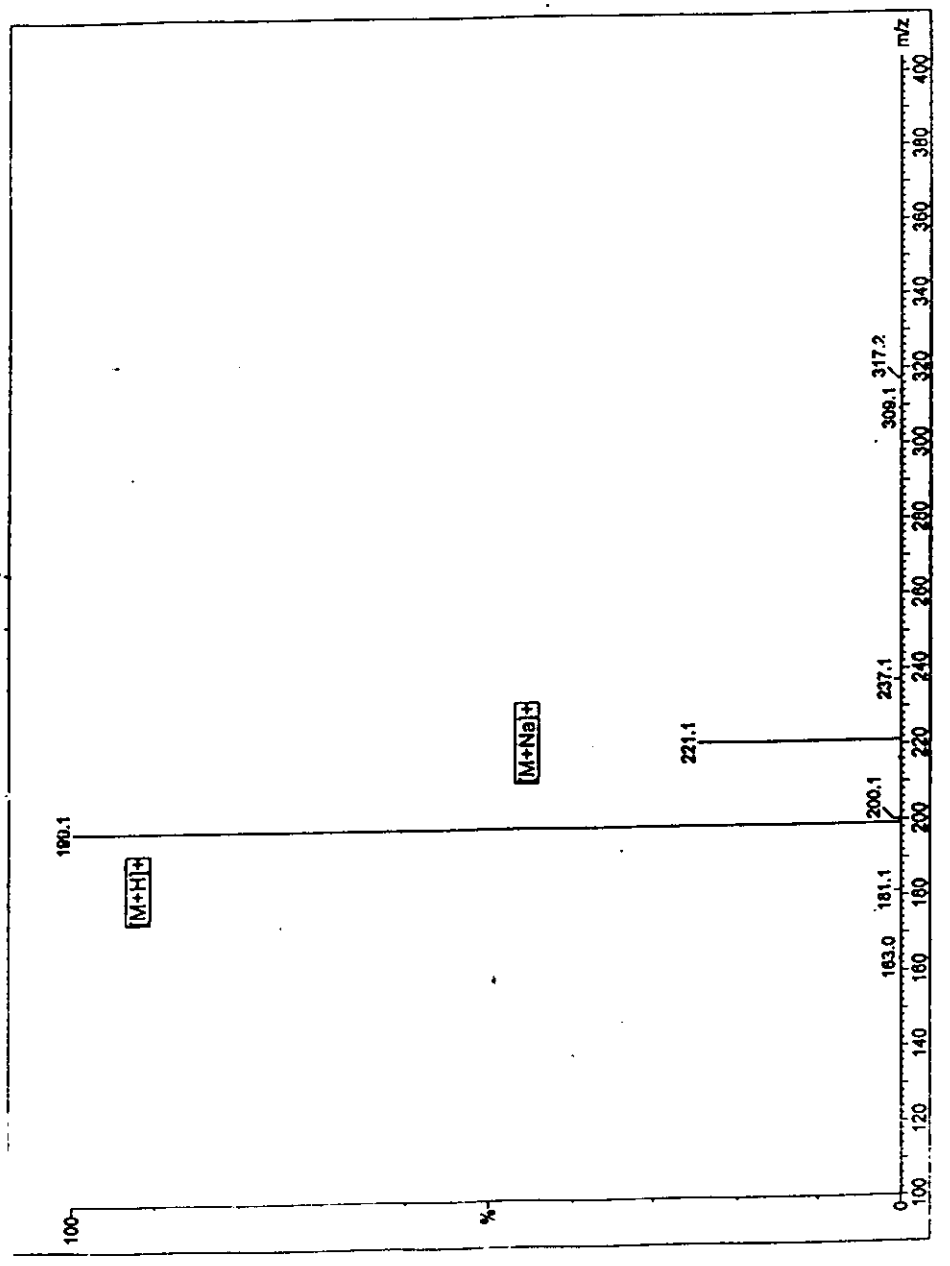


Figure 100 Mass spectrum of VR-JOY8

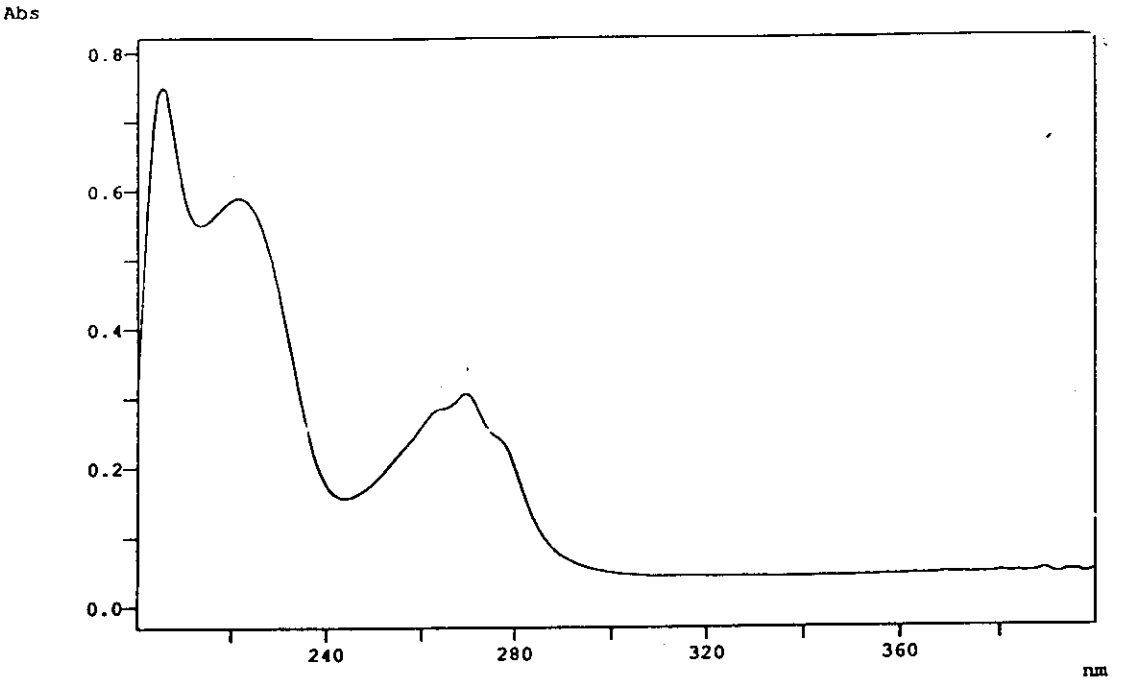


Figure 101 UV (MeOH) spectrum of VR-JOY14

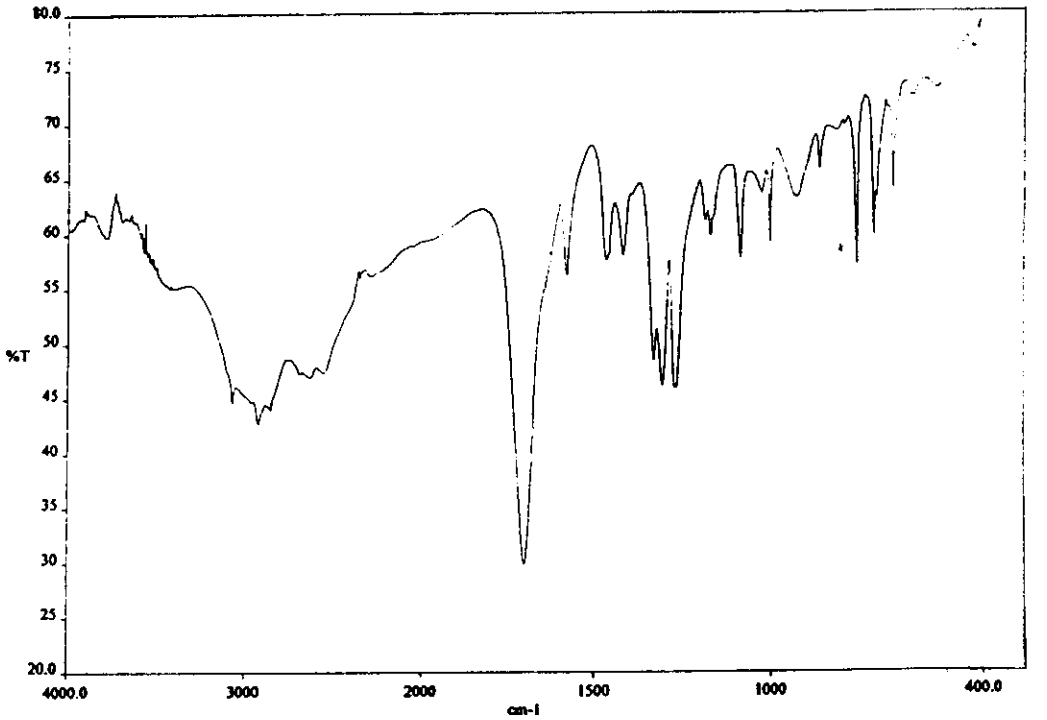


Figure 102 FT-IR (KBr) spectrum of VR-JOY14

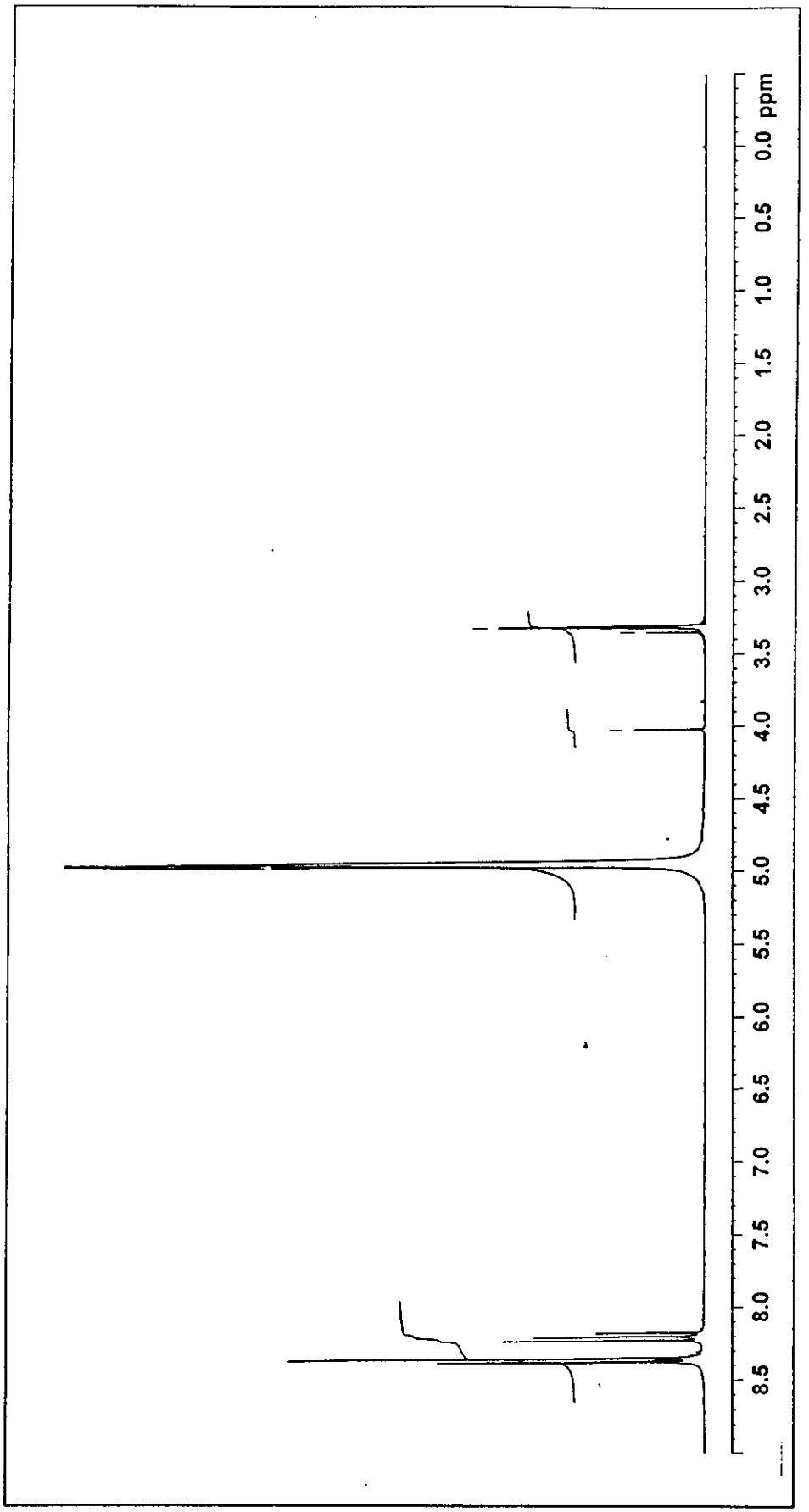


Figure 103 ^{1}H NMR (300 MHz) (CD_3OD) spectrum of VR-JOY14

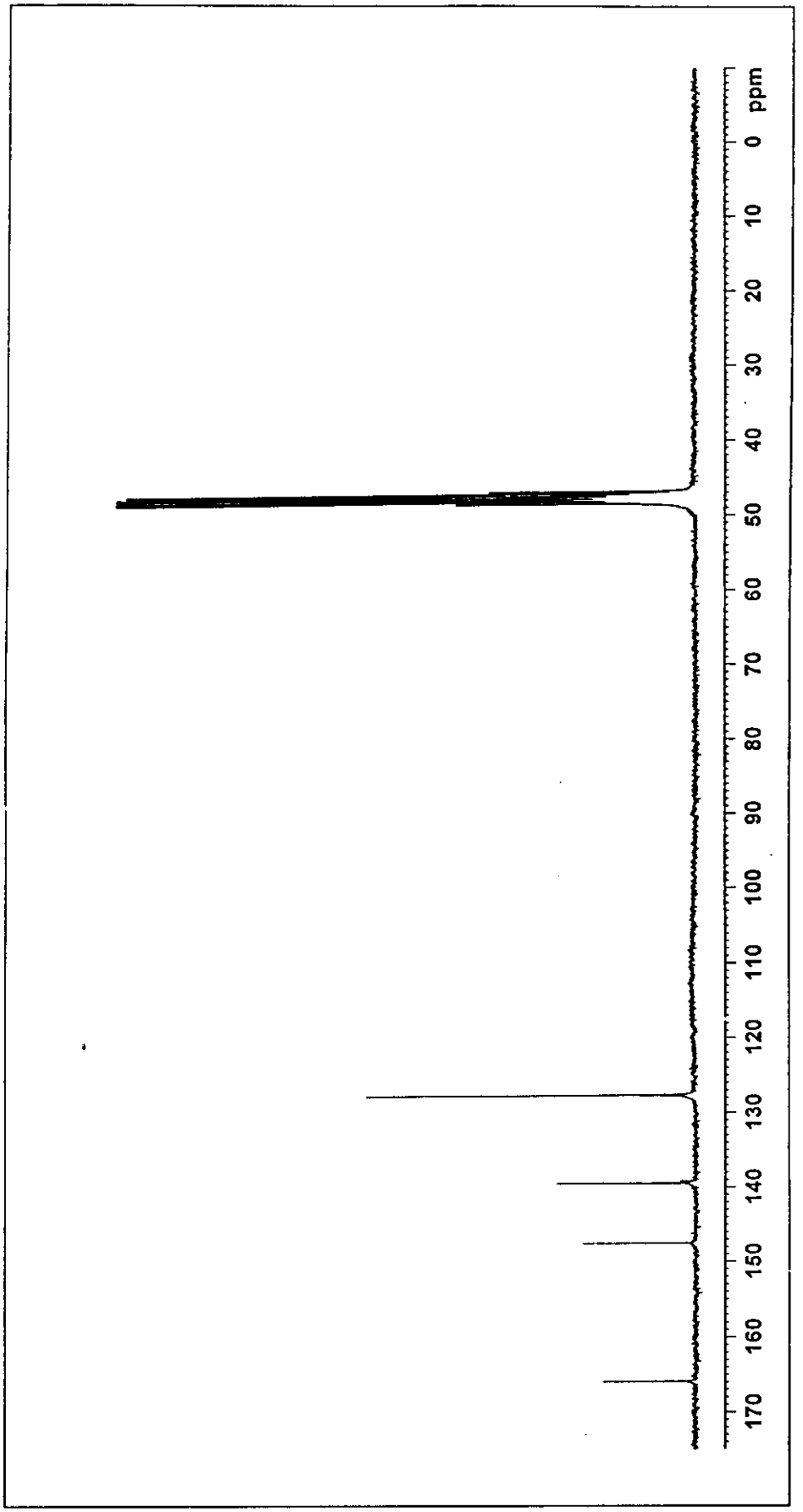


Figure 104 ^{13}C NMR (75 MHz) (CD_3OD) spectrum of VR-JOY14

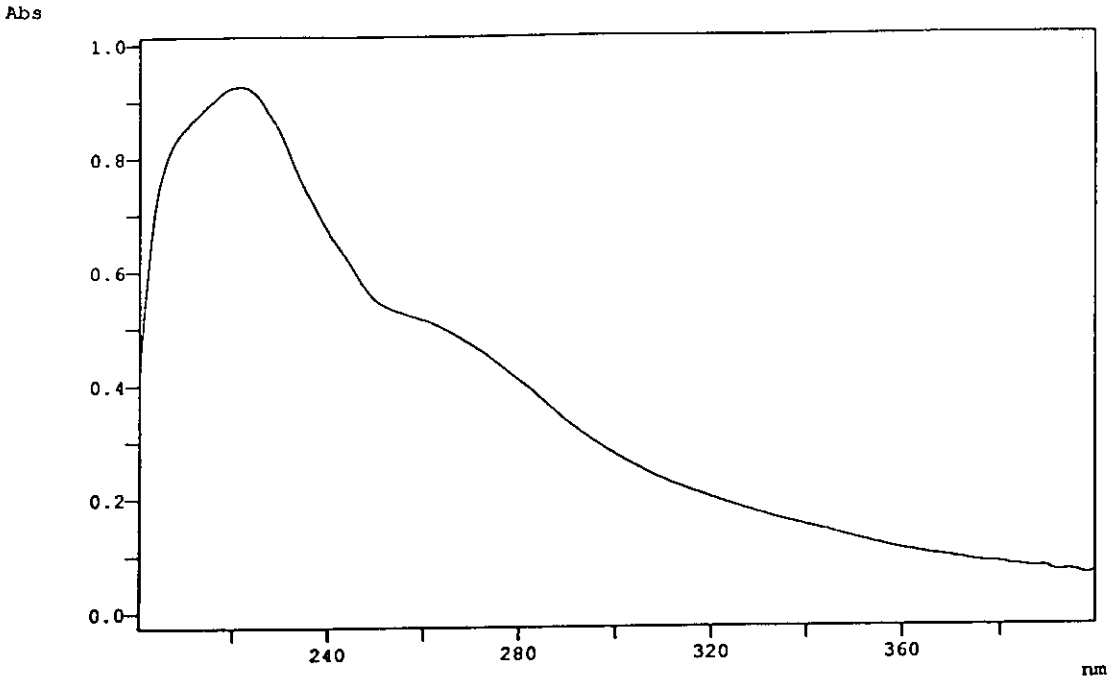


Figure 105 UV (MeOH) spectrum of VR-JOY15

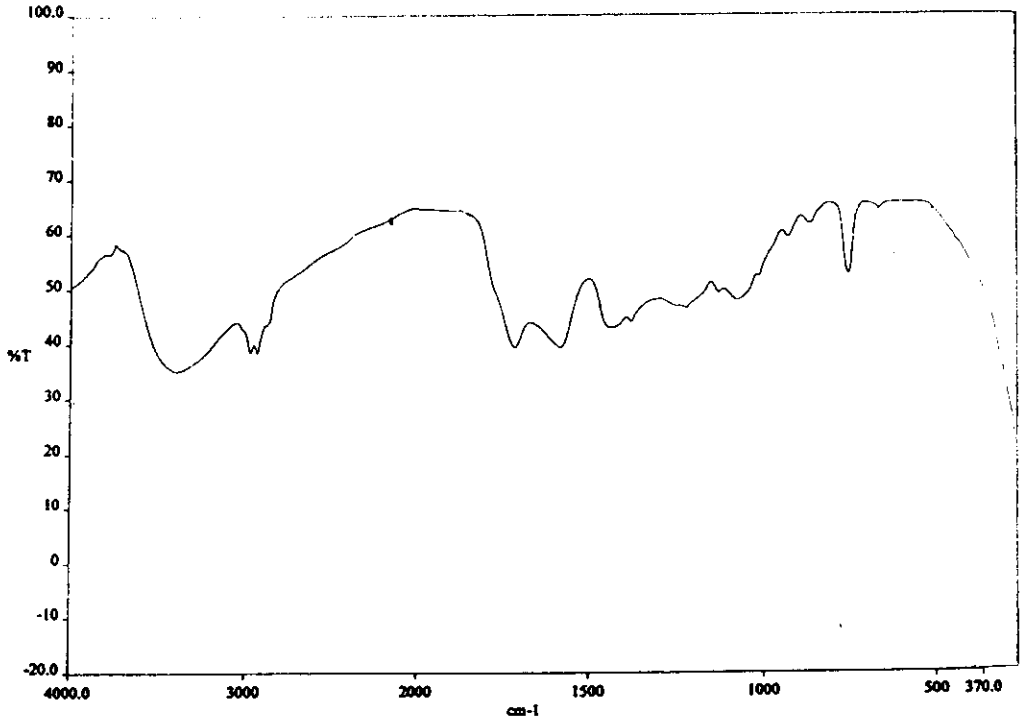


Figure 106 FT-IR (neat) spectrum of VR-JOY15

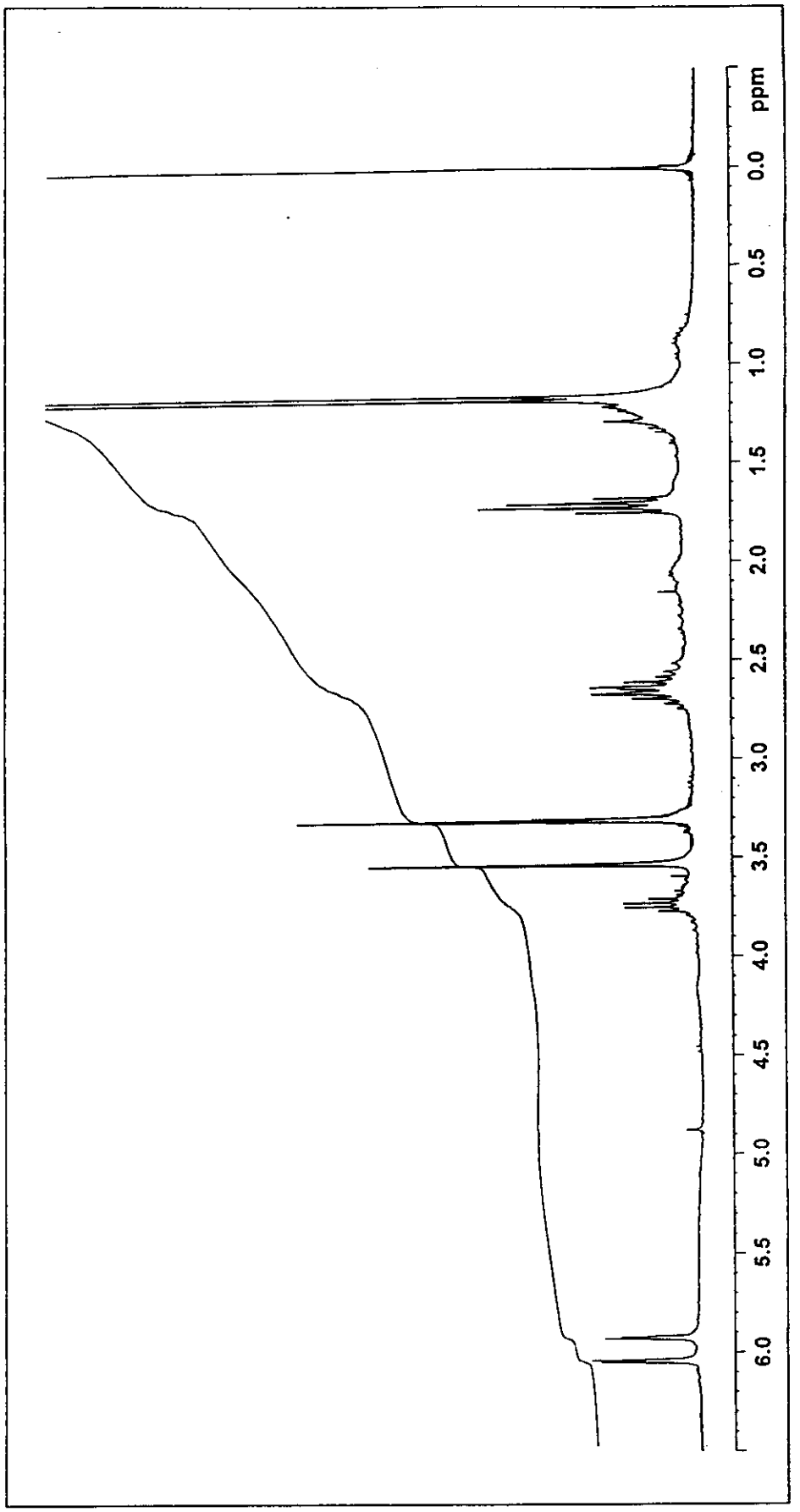


Figure 107 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of VR-JOY15

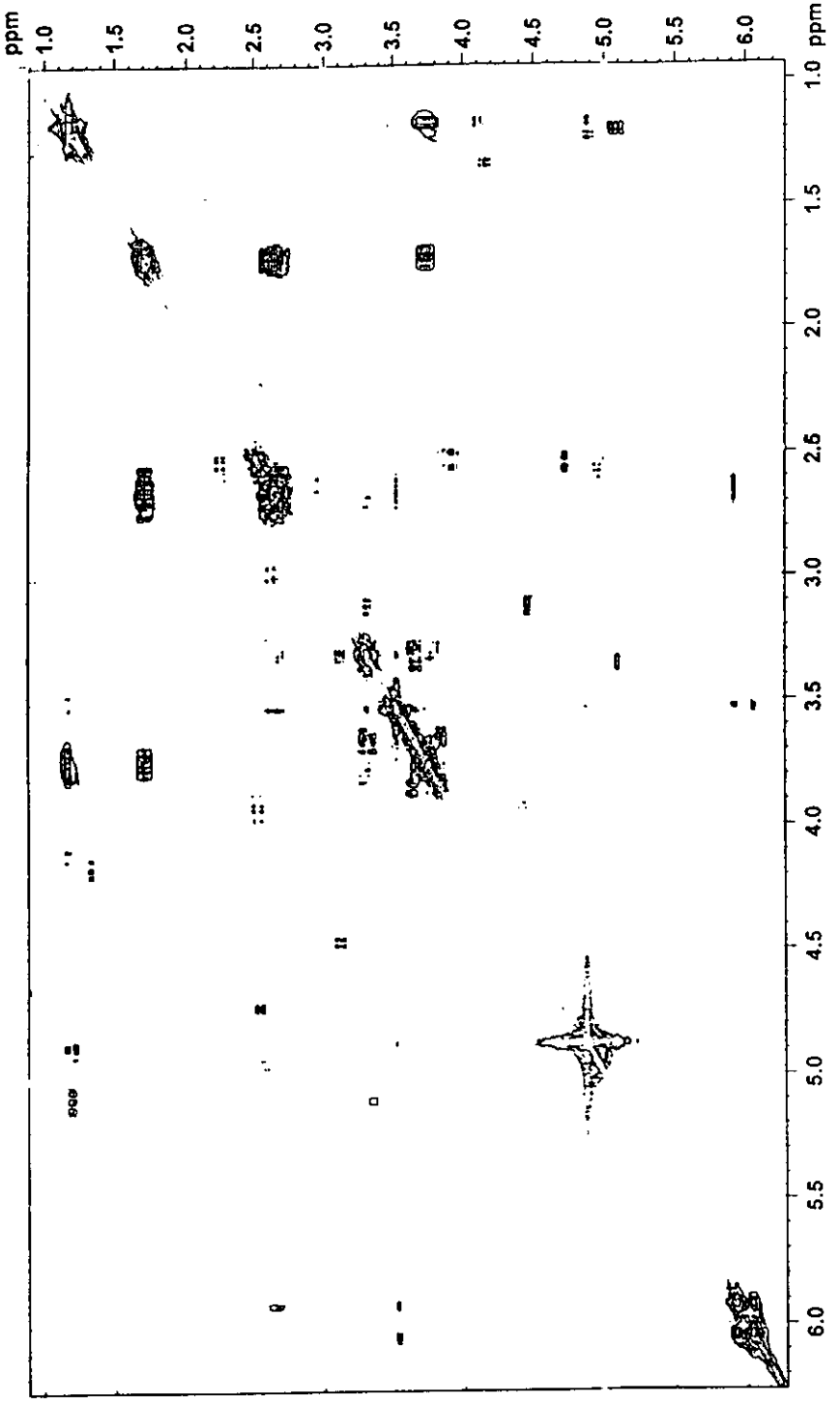


Figure 108 COSY (300 MHz) spectrum of VR-JOY15

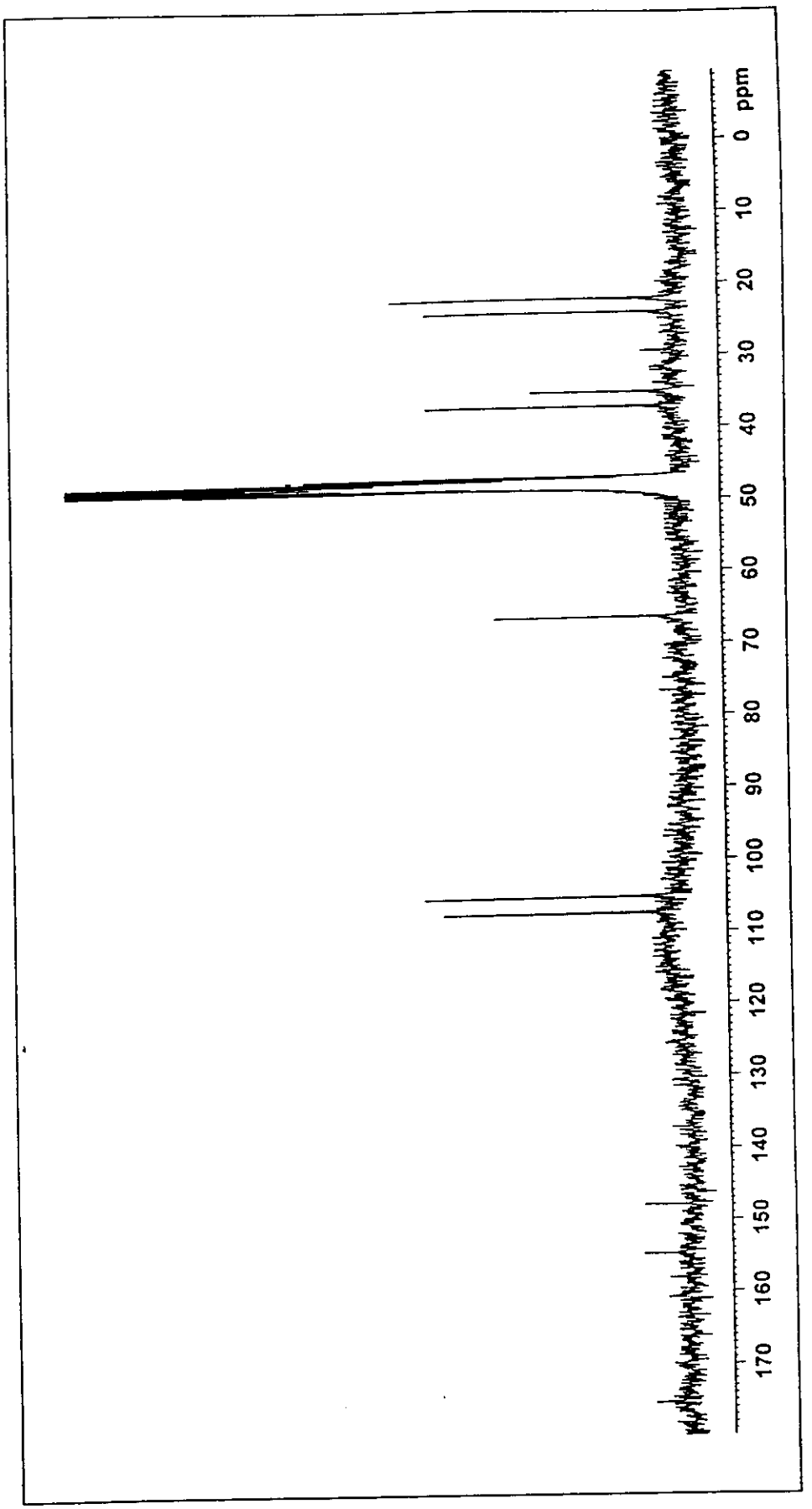


Figure 109 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of VR-JOY15

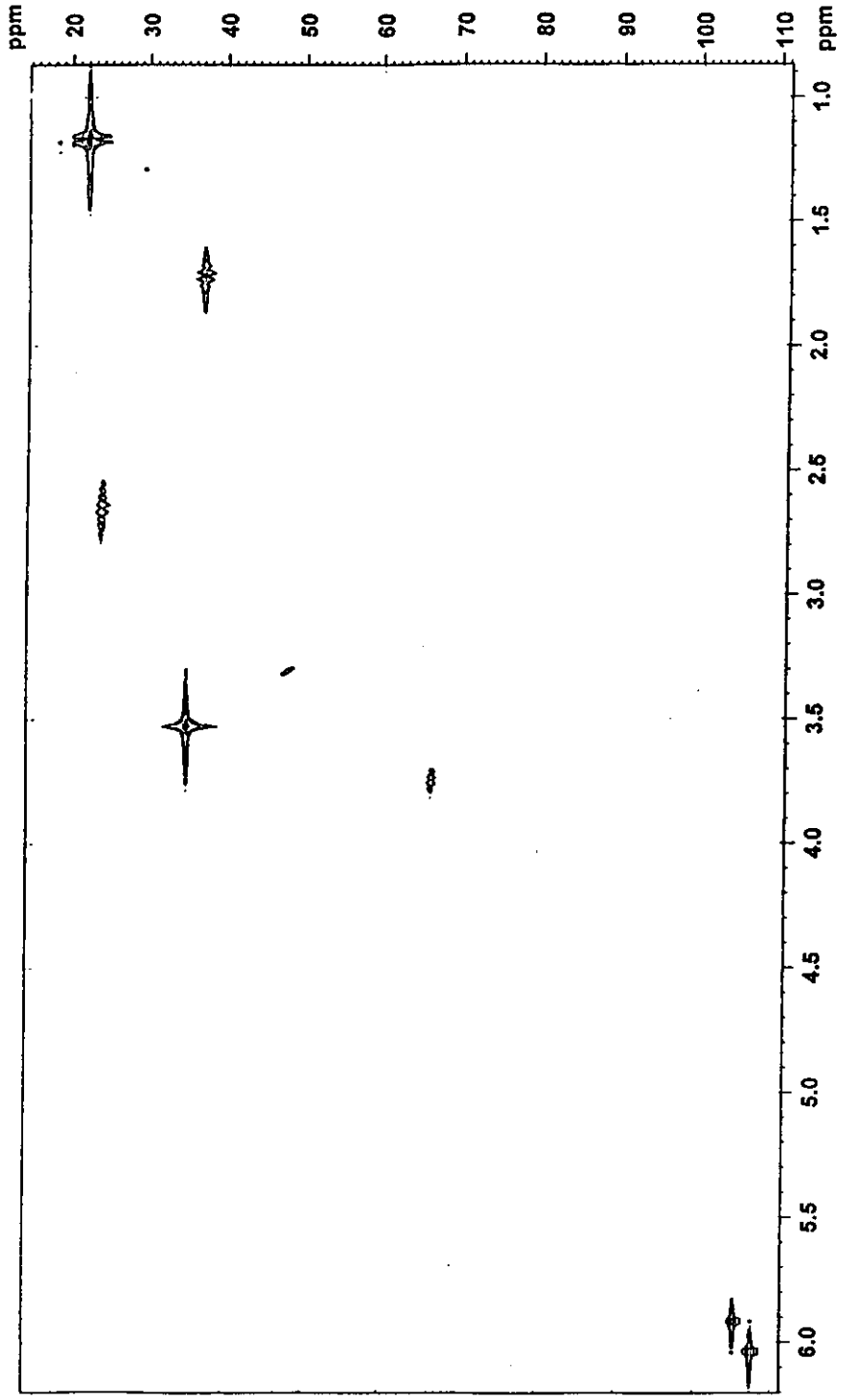


Figure 110 2D HMQC (300 MHz) spectrum of VR-JOY15

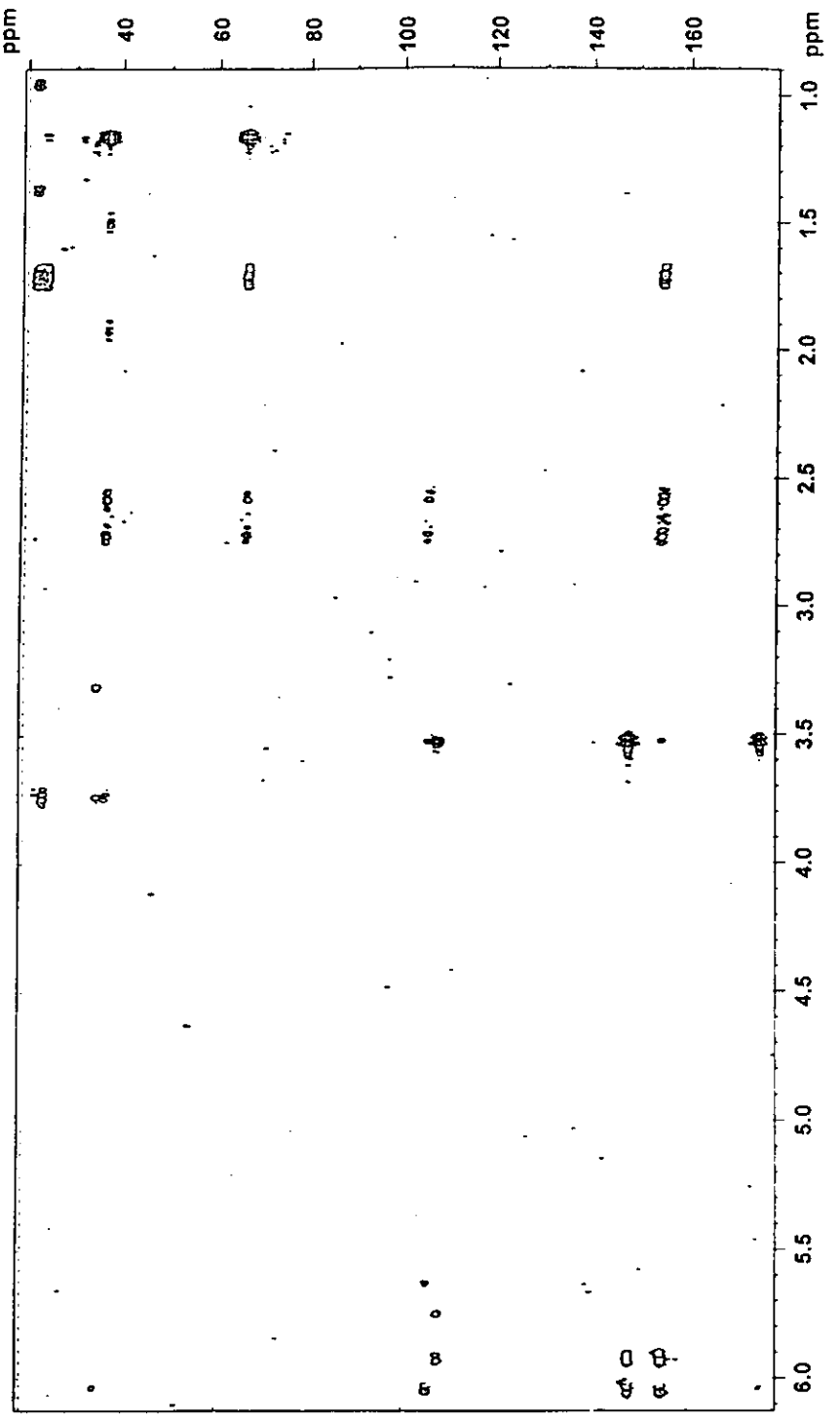


Figure 111 2D HMBC (300 MHz) spectrum of VR-JOY15