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APPENDIX

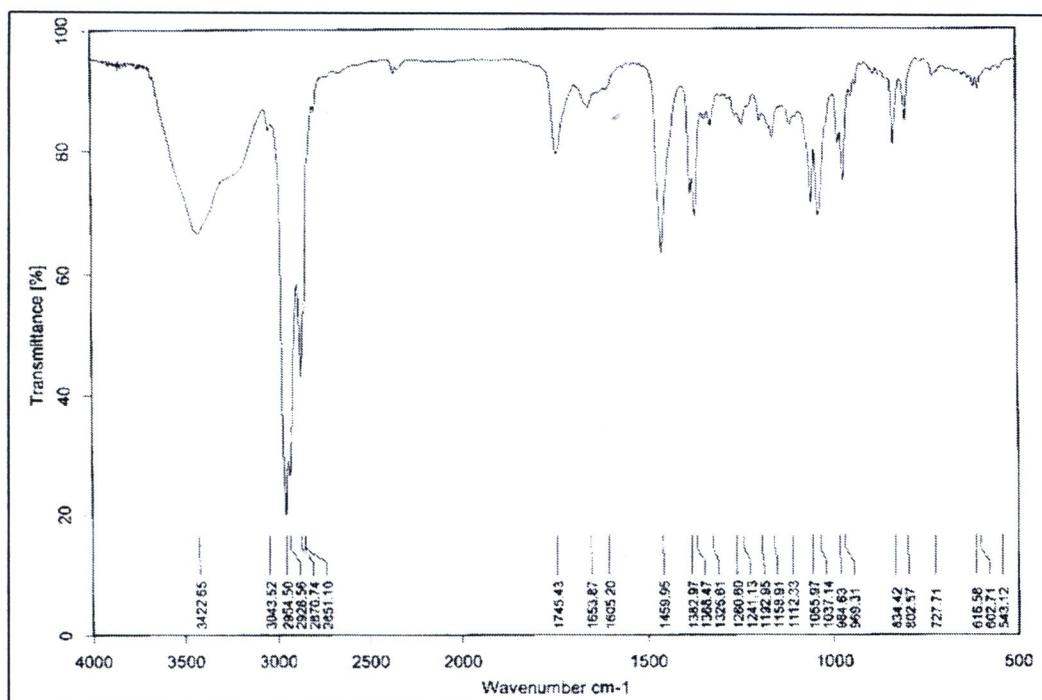


Figure 1 IR spectrum of ergosterol (I).

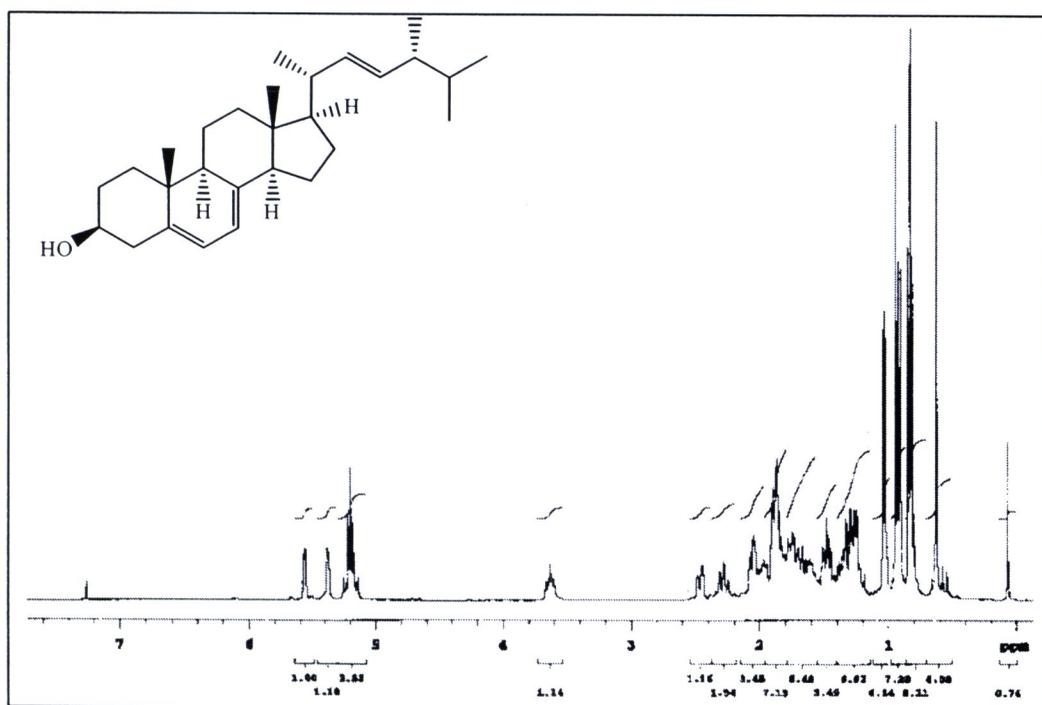


Figure 2 ^1H NMR spectrum of ergosterol (I).

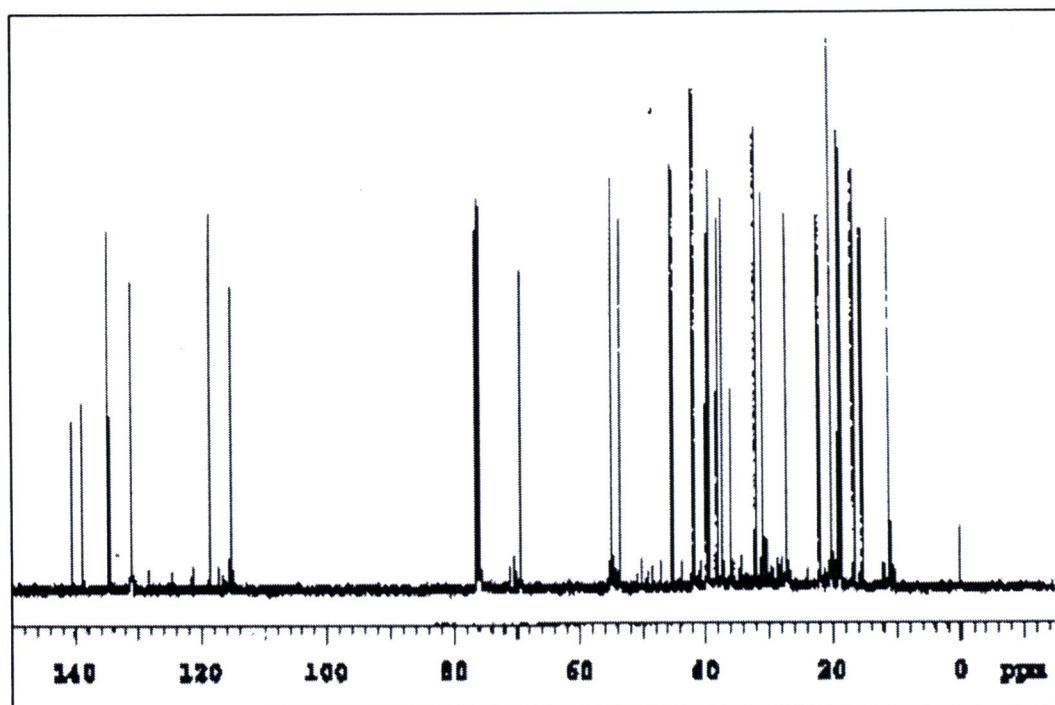


Figure 3 ^{13}C NMR spectrum of ergosterol (I).

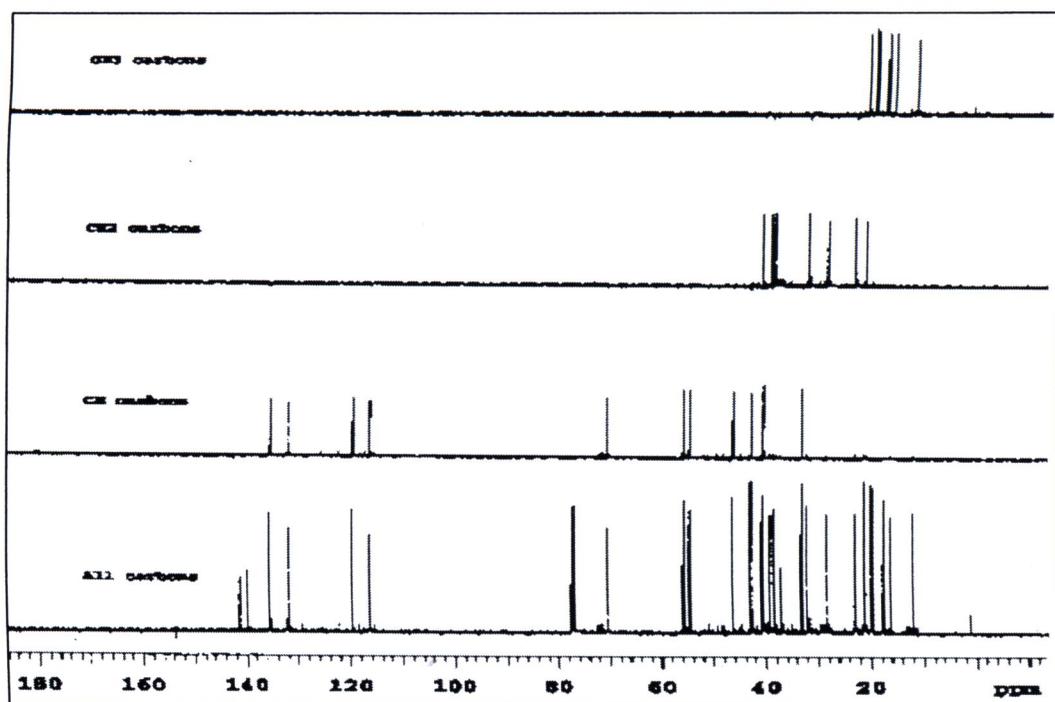


Figure 4 DEPT spectrum of ergosterol (I).

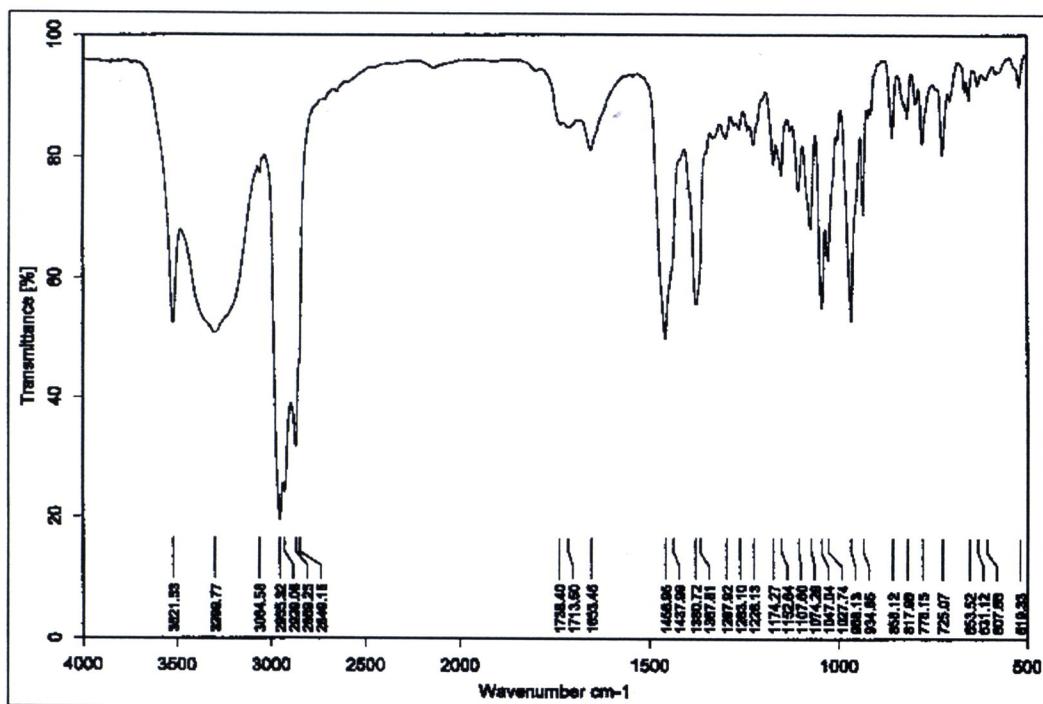


Figure 5 IR spectrum of ergosterol peroxide (II).

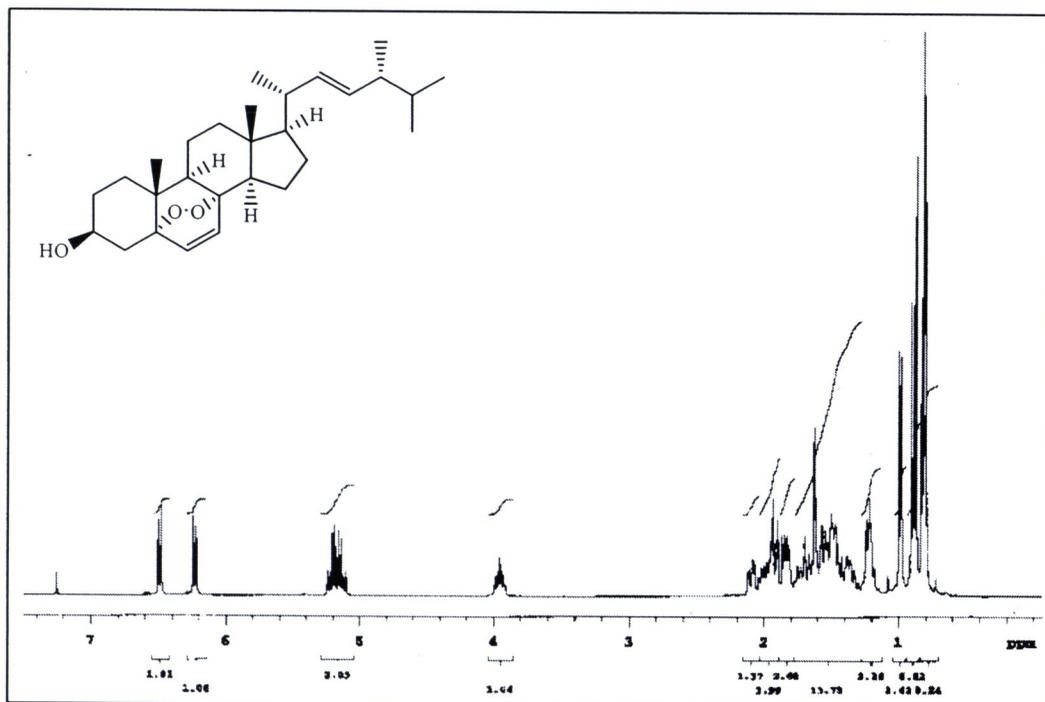


Figure 6 ^1H NMR spectrum of ergosterol peroxide (II).

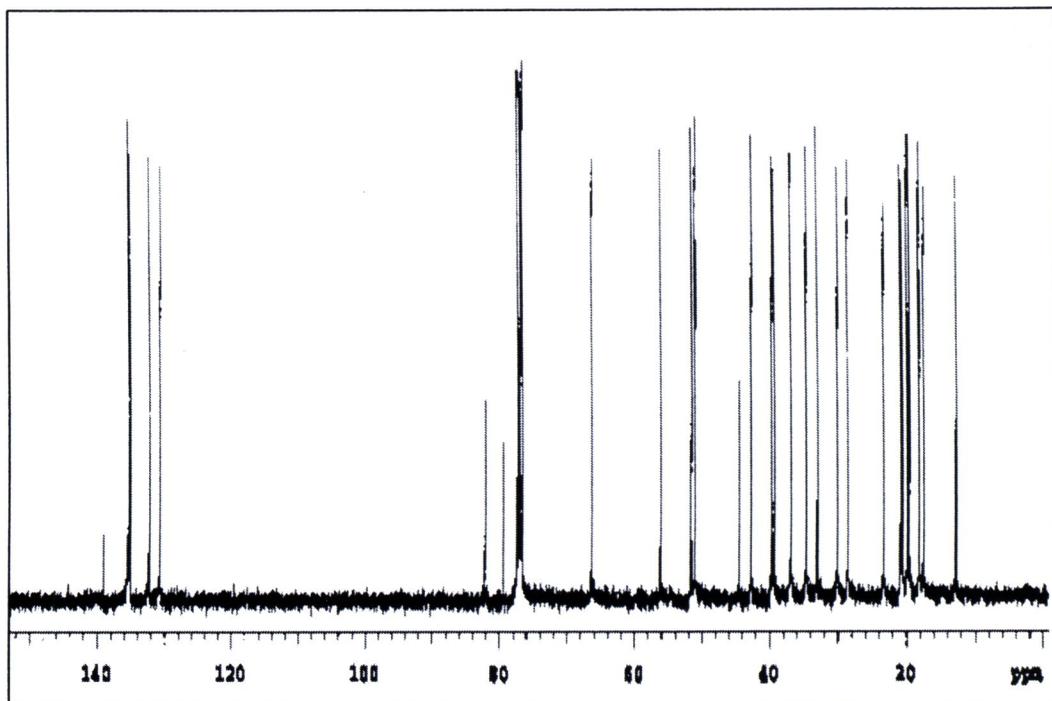


Figure 7 ^{13}C spectrum of ergosterol peroxide (II).

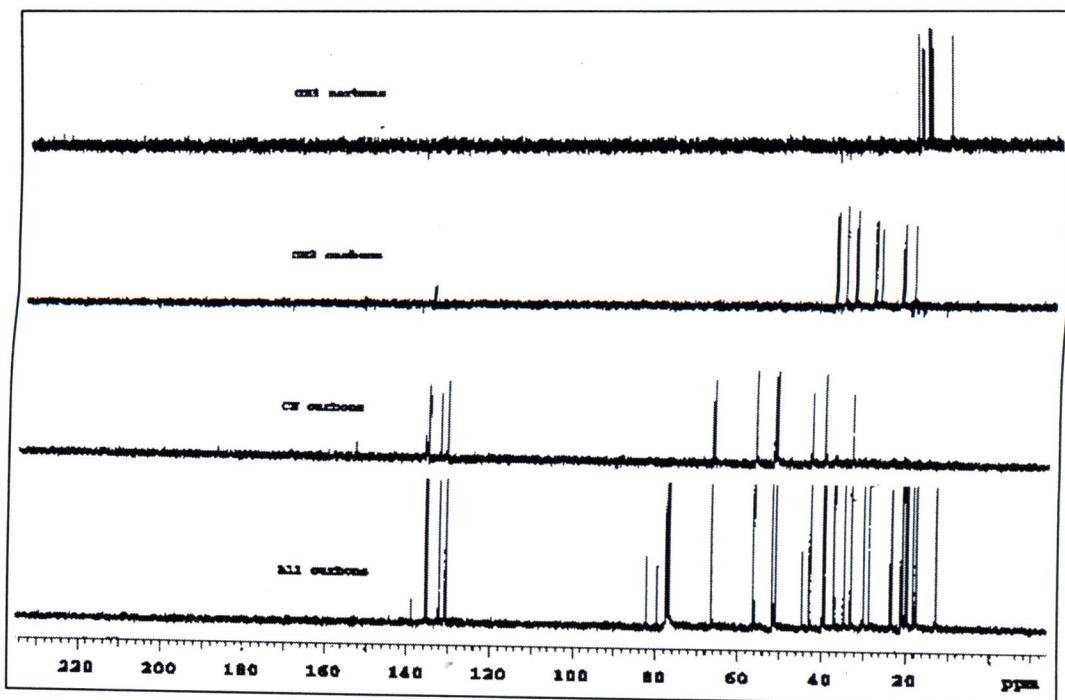


Figure 8 DEPT spectrum of ergosterol peroxide (II).

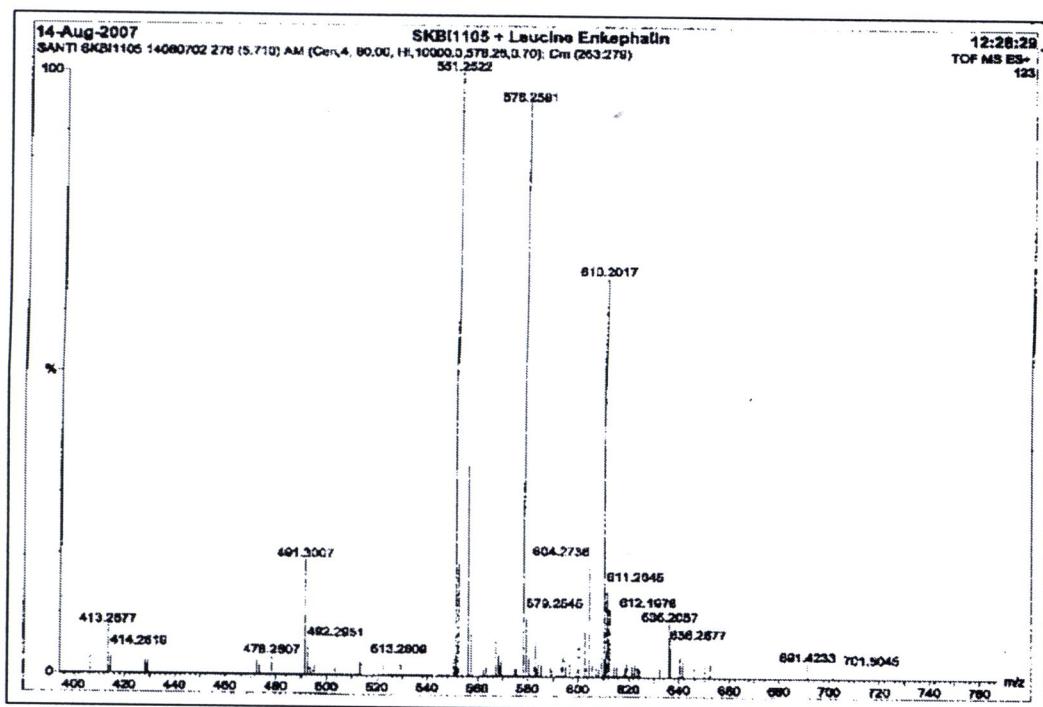


Figure 9 Mass spectrum of chaetoglobosin C (III).

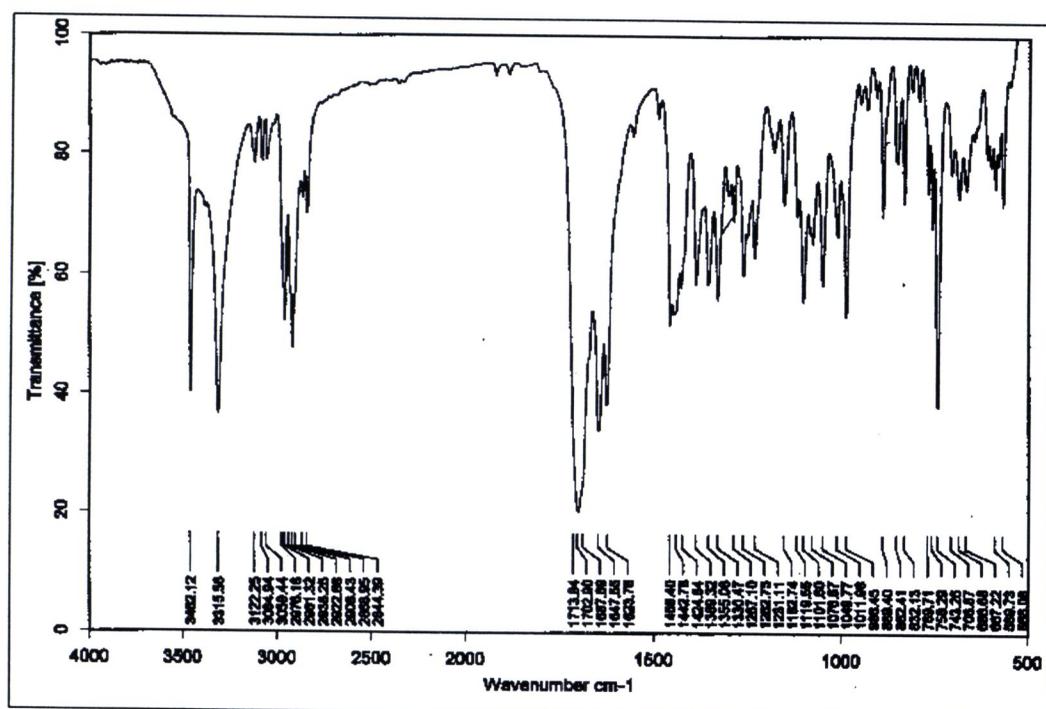


Figure 10 IR spectrum of chaetoglobosin C (III).

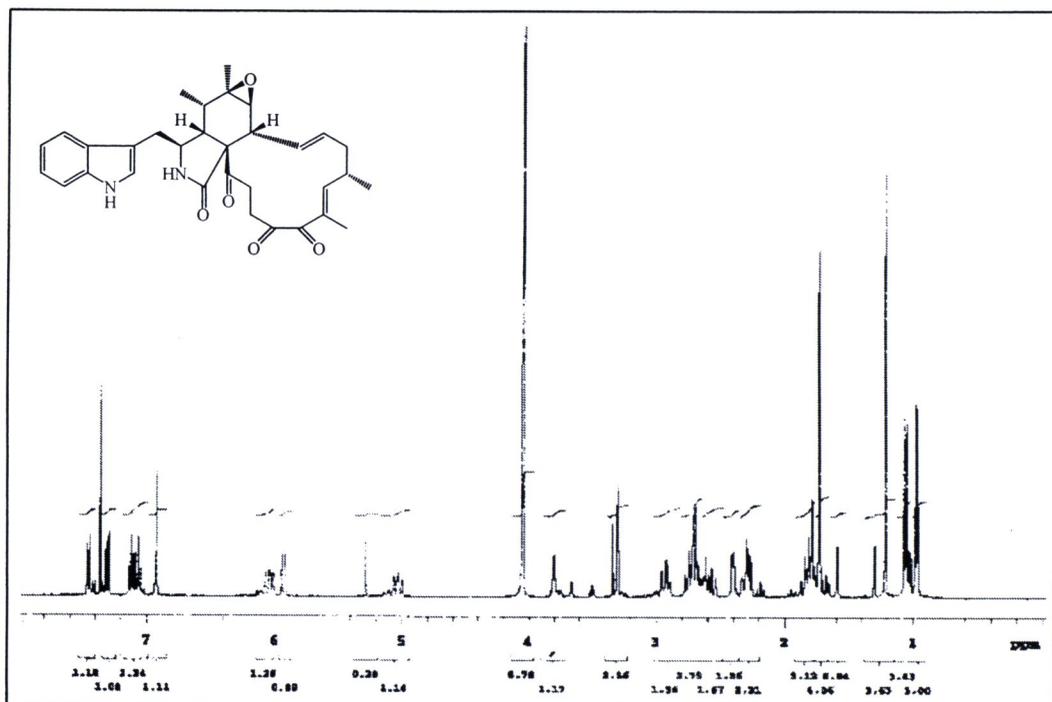


Figure 11 ^1H NMR spectrum of chaetoglobosin C (III).

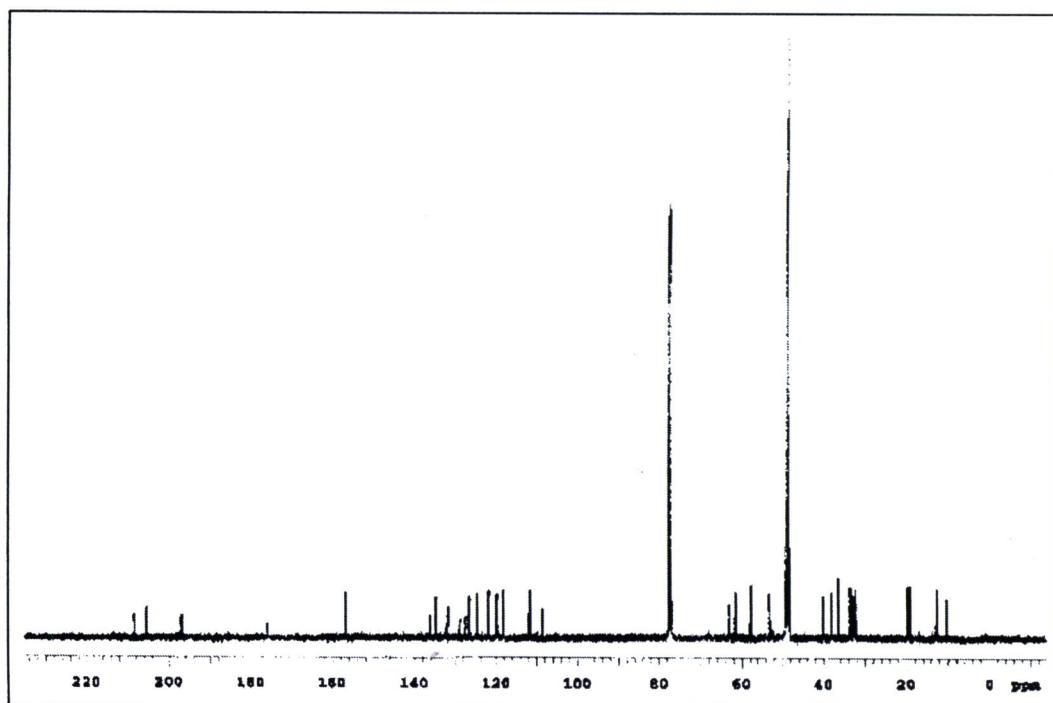


Figure 12 ^{13}C NMR spectrum of chaetoglobosin C (III).

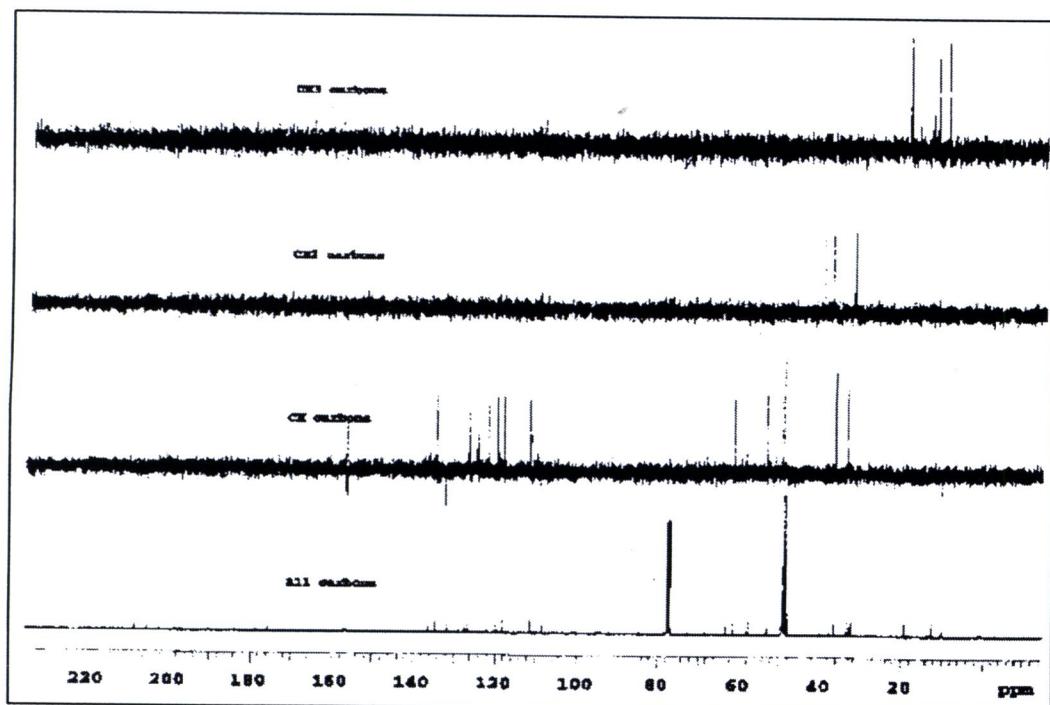


Figure 13 DEPT spectrum of chaetoglobosin C (III).

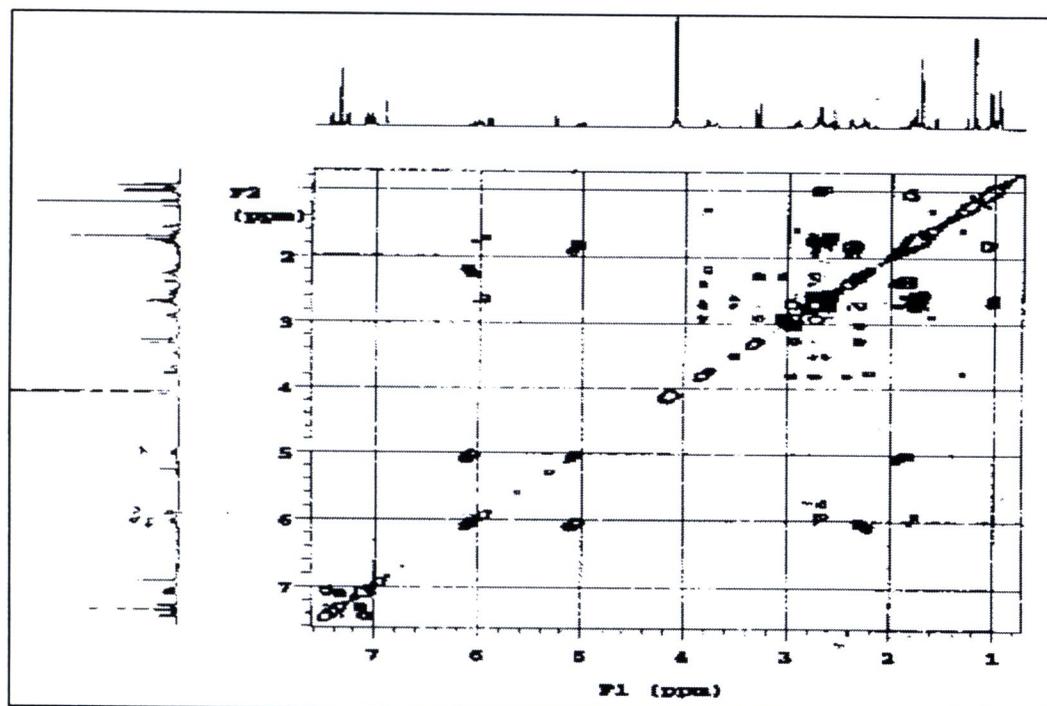


Figure 14 COSY spectrum of chaetoglobosin C (III).

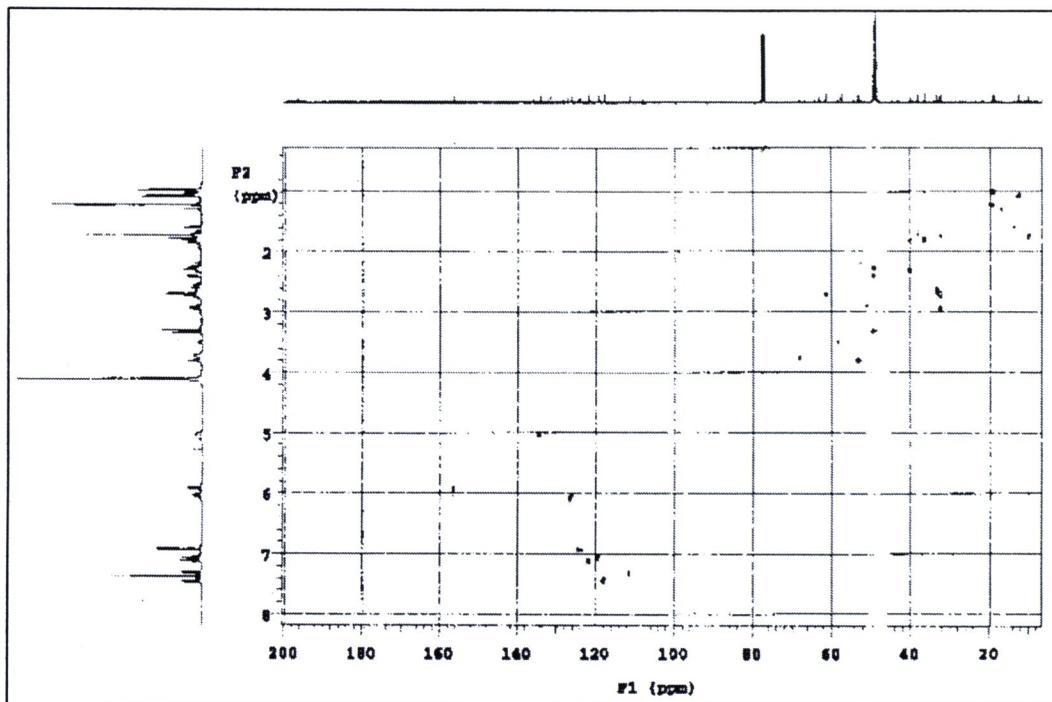


Figure 15 HSQC spectrum of chaetoglobosin C (III).

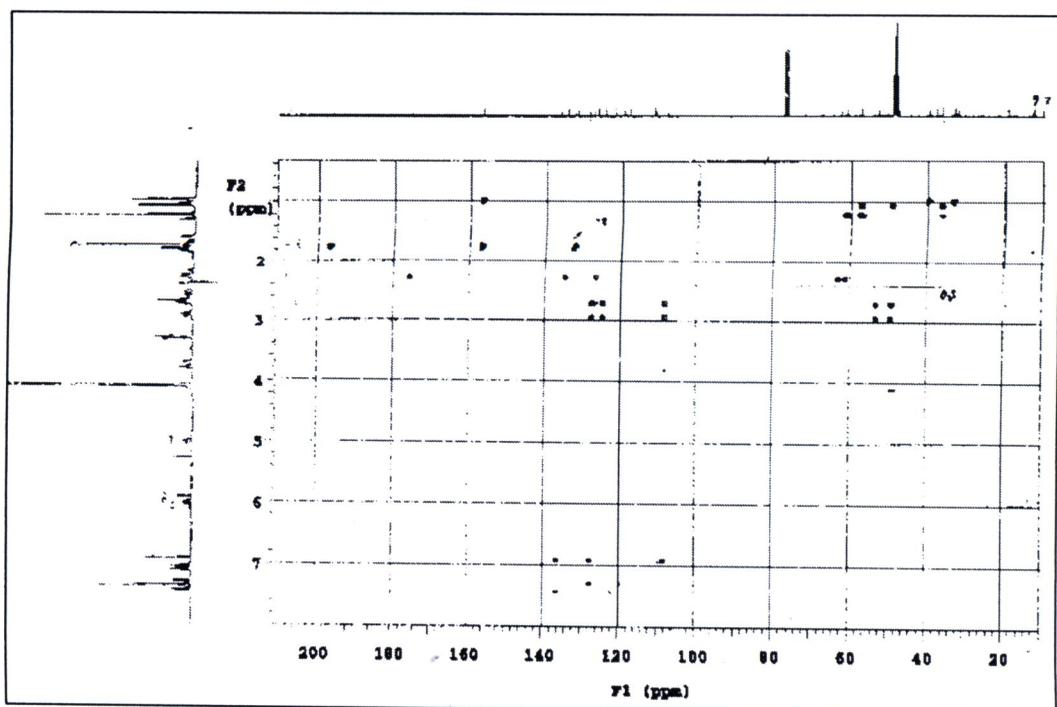


Figure 16 HMBC spectrum of chaetoglobosin C (III).

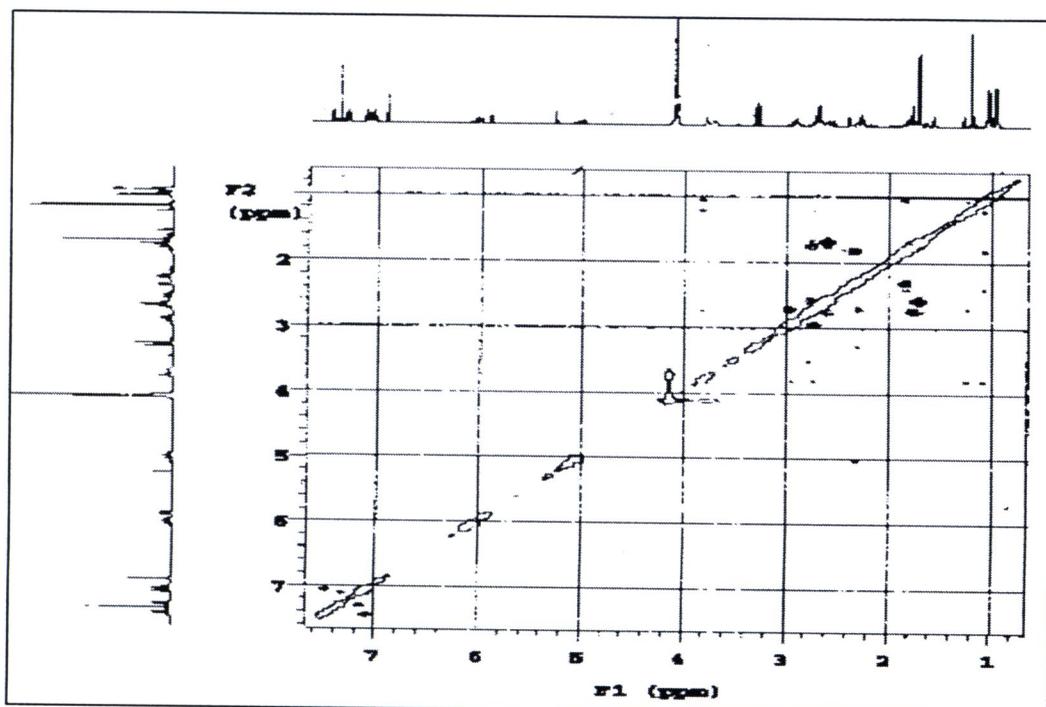


Figure 17 NOESY spectrum of chaetoglobosin C (III).

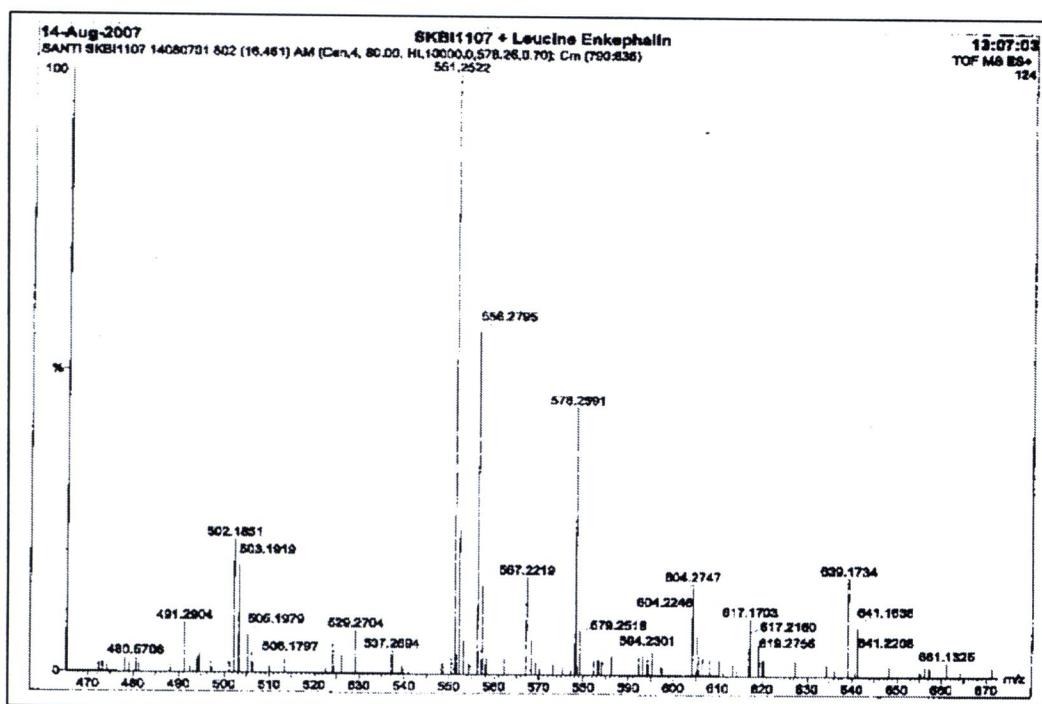


Figure 18 Mass spectrum of isochaetoglobosin D (IV).

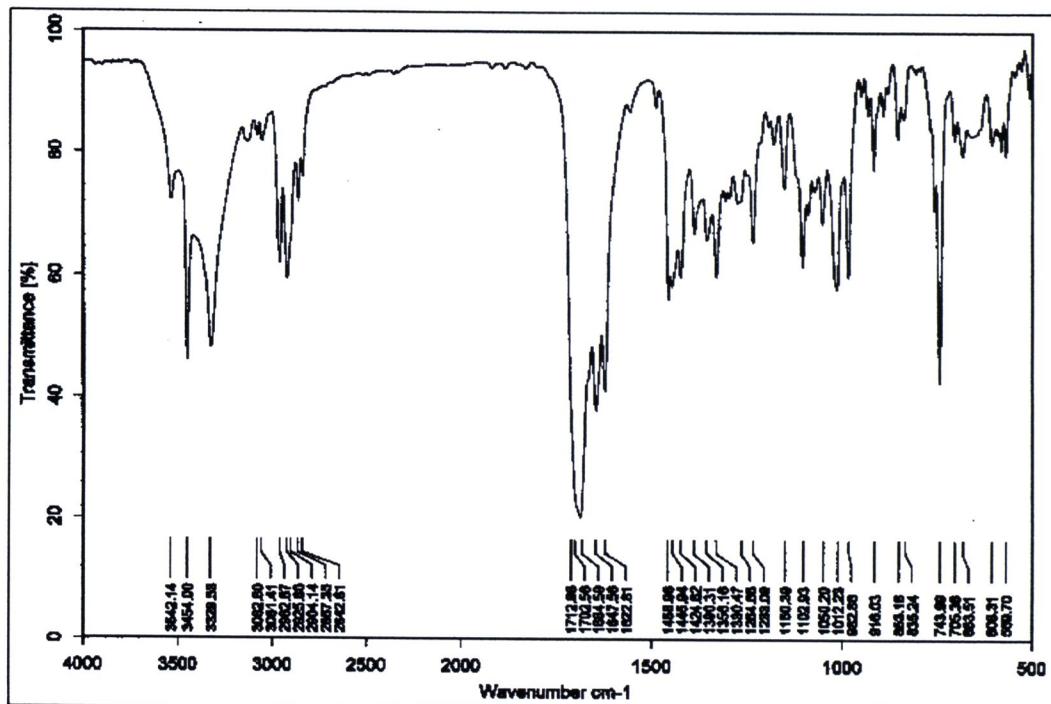


Figure 19 IR spectrum of isochaetoglobosin D (IV).

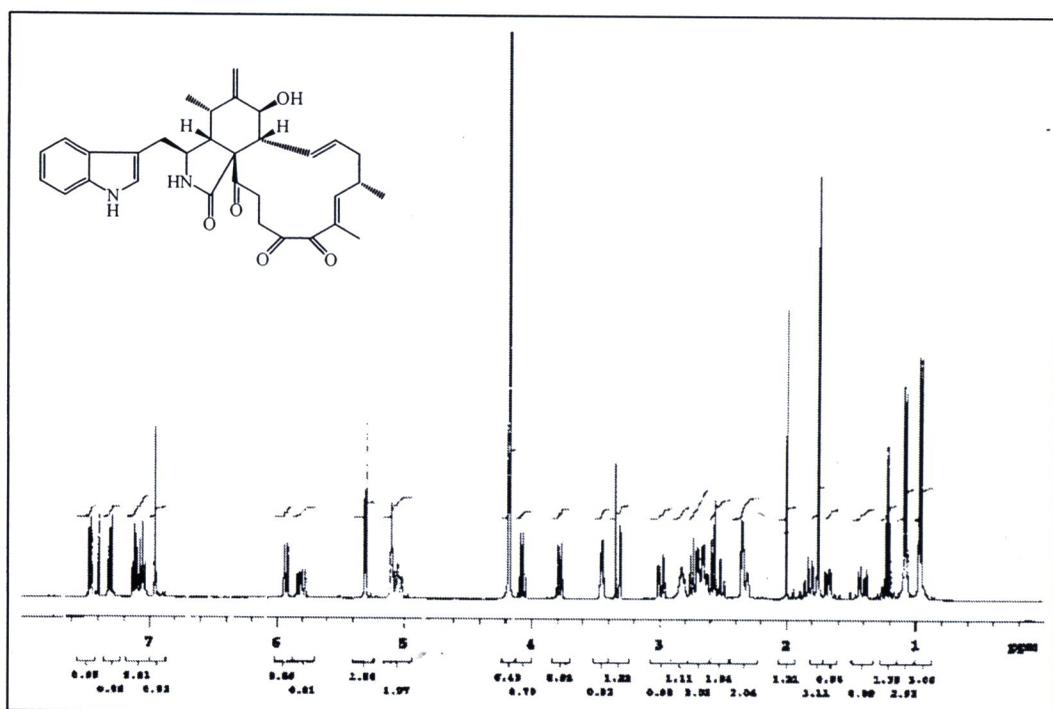


Figure 20 ¹H NMR spectrum of isochaetoglobosin D (IV).

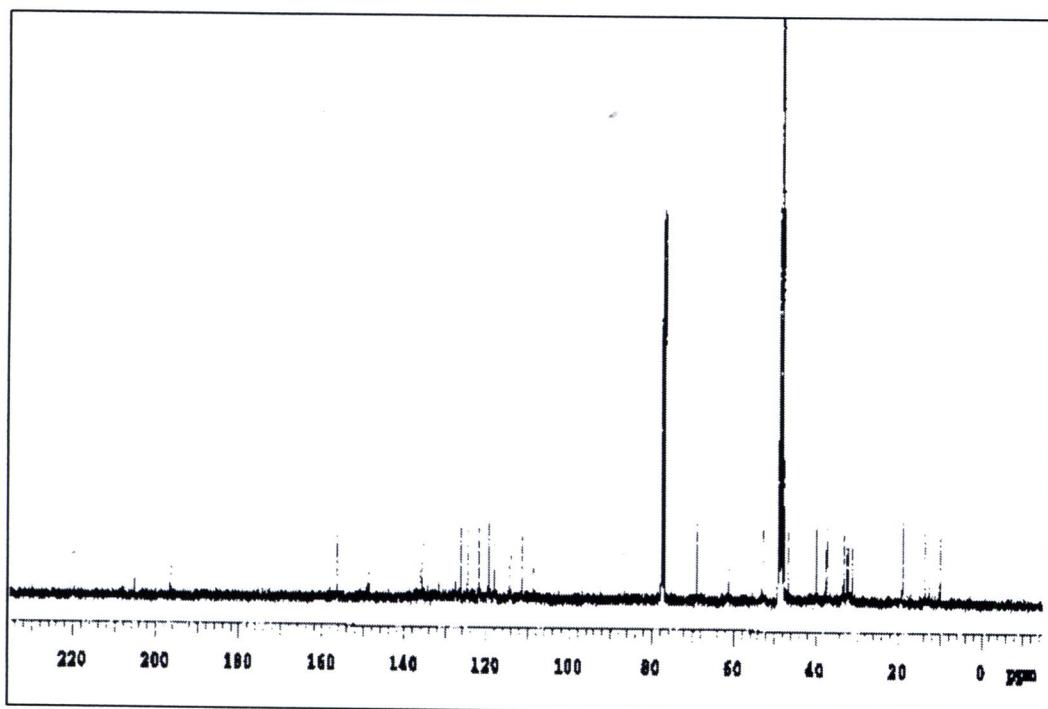


Figure 21 ^{13}C NMR spectrum of isochaetoglobosin D (IV).

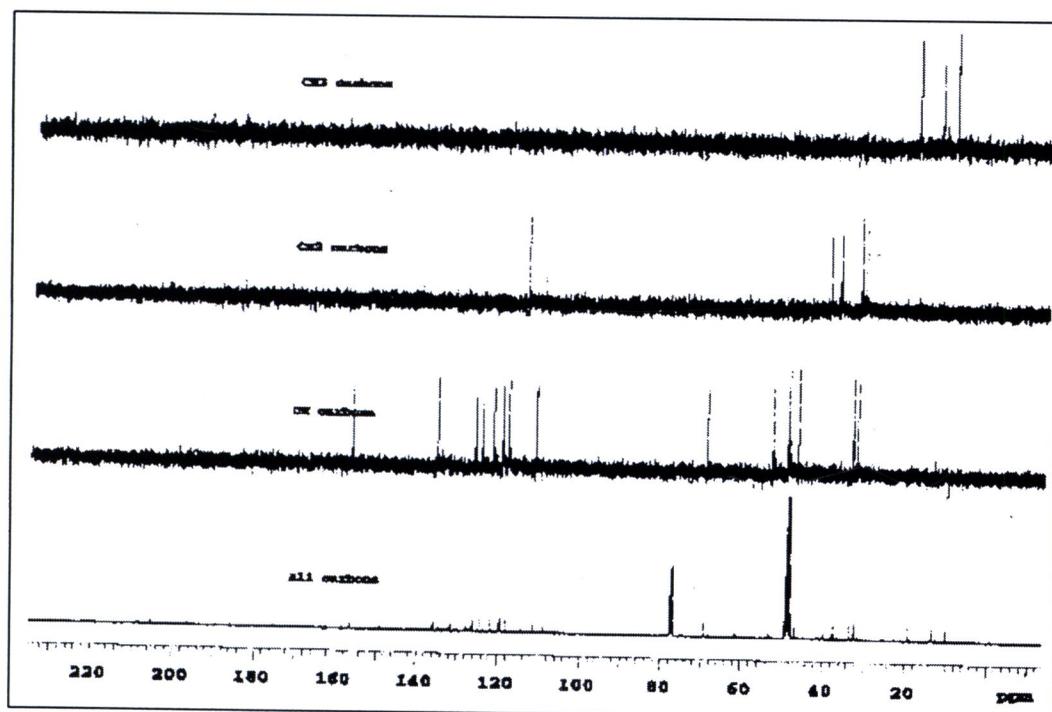


Figure 22 DEPT spectrum of isochaetoglobosin D (IV).

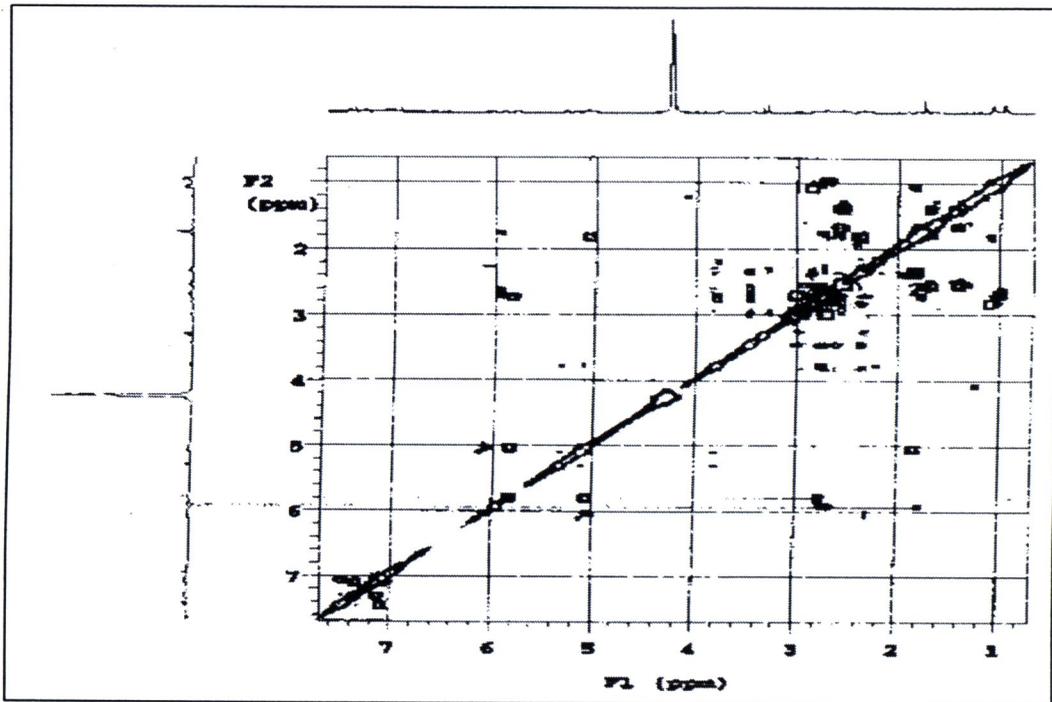


Figure 23 COSY spectrum of isochaetoglobosin D (IV).

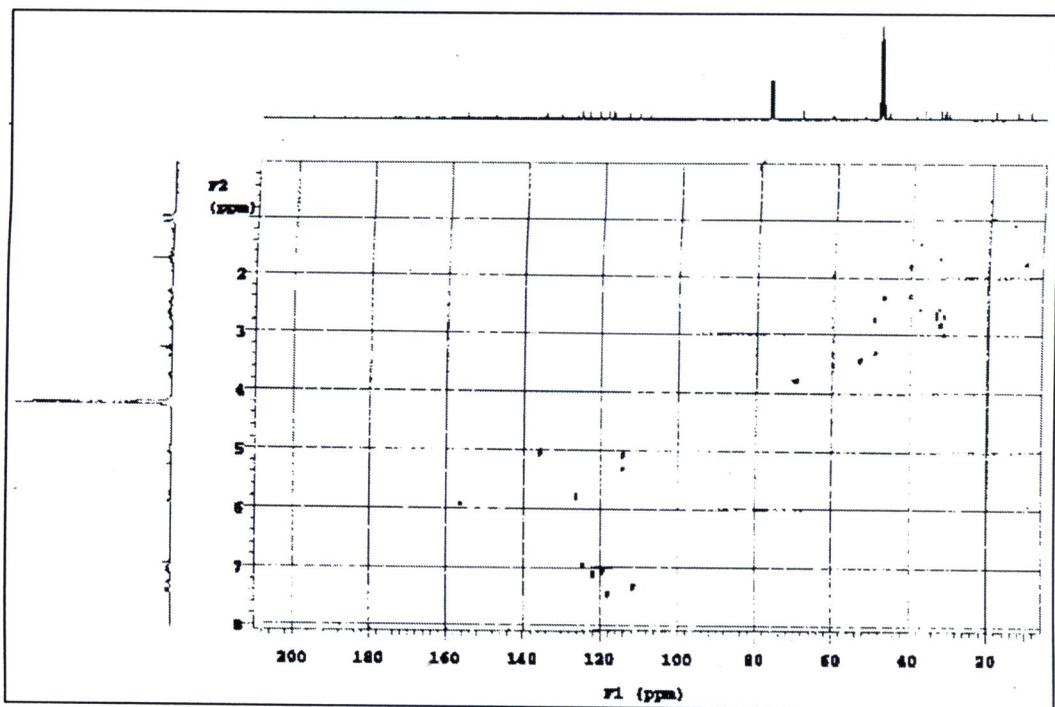


Figure 24 HSQC spectrum of isochaetoglobosin D (IV).

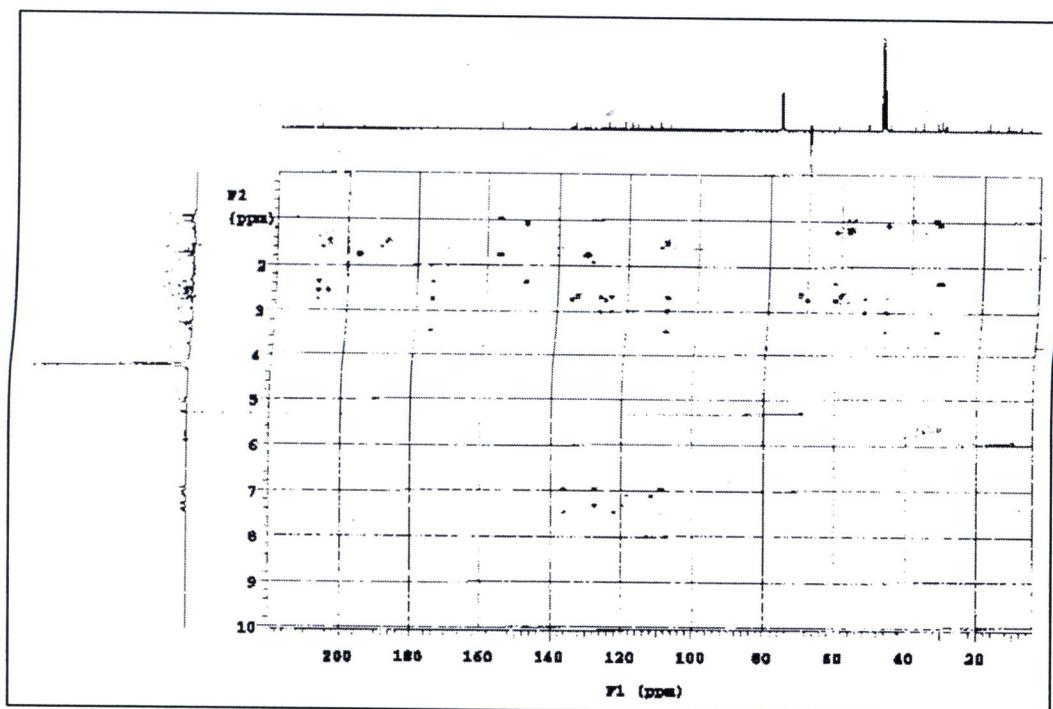


Figure 25 HMBC spectrum of isochaetoglobosin D (IV).

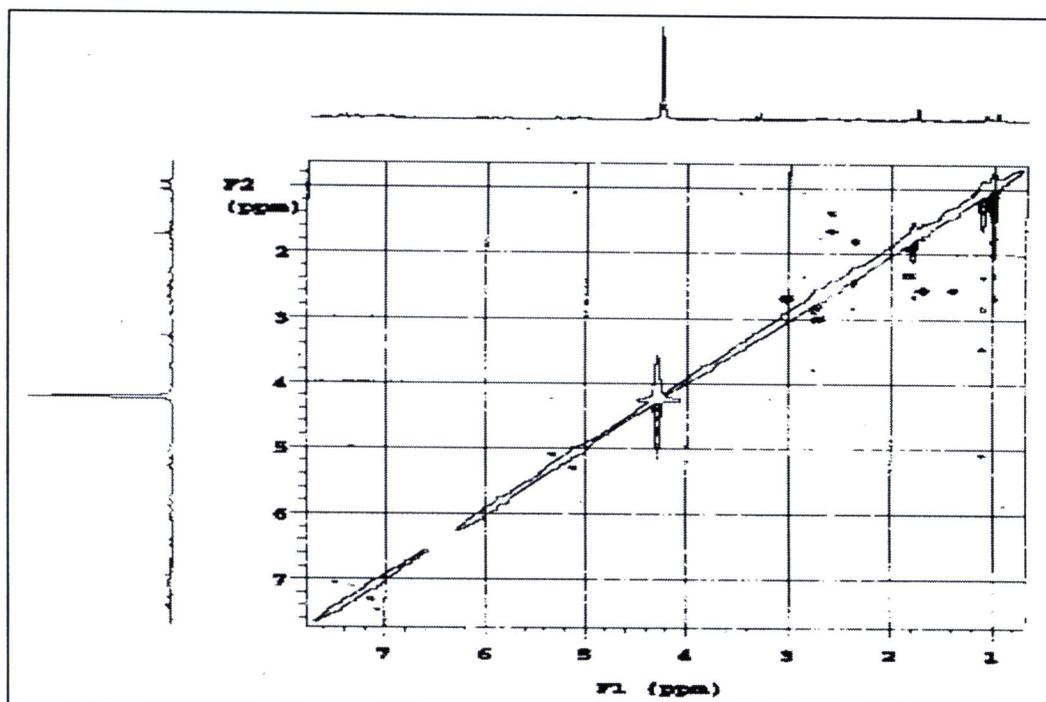


Figure 26 NOESY spectrum of isochaetoglobosin D (IV).

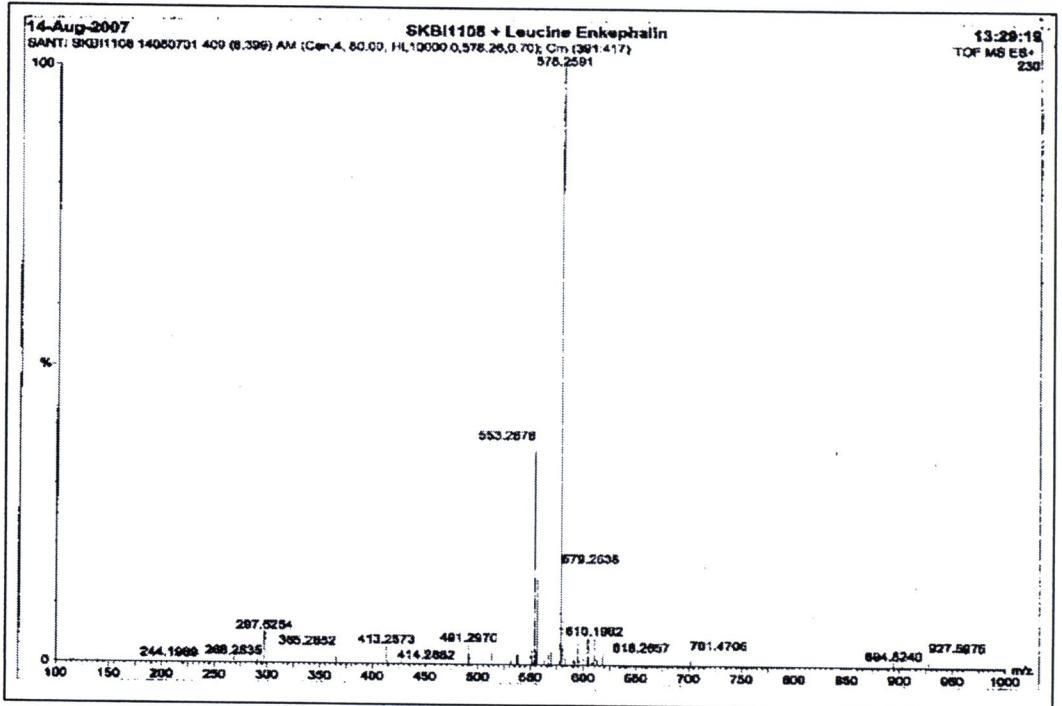


Figure 27 Mass spectrum of chaetoglobosin F (V).

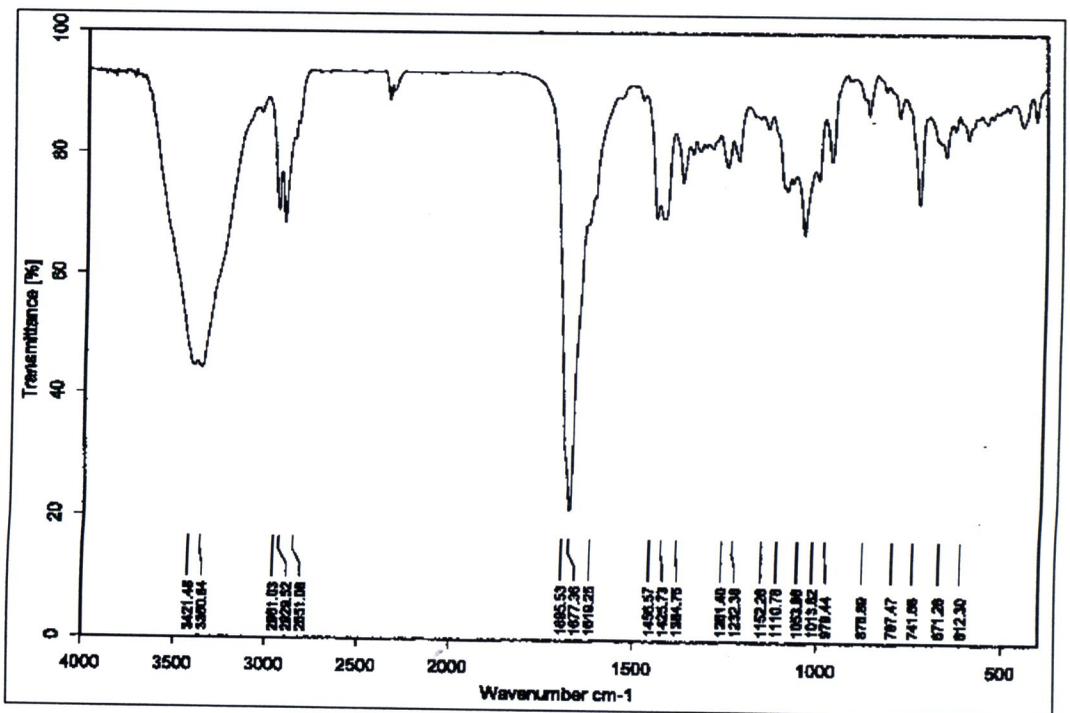


Figure 28 IR spectrum of chaetoglobosin F (V).

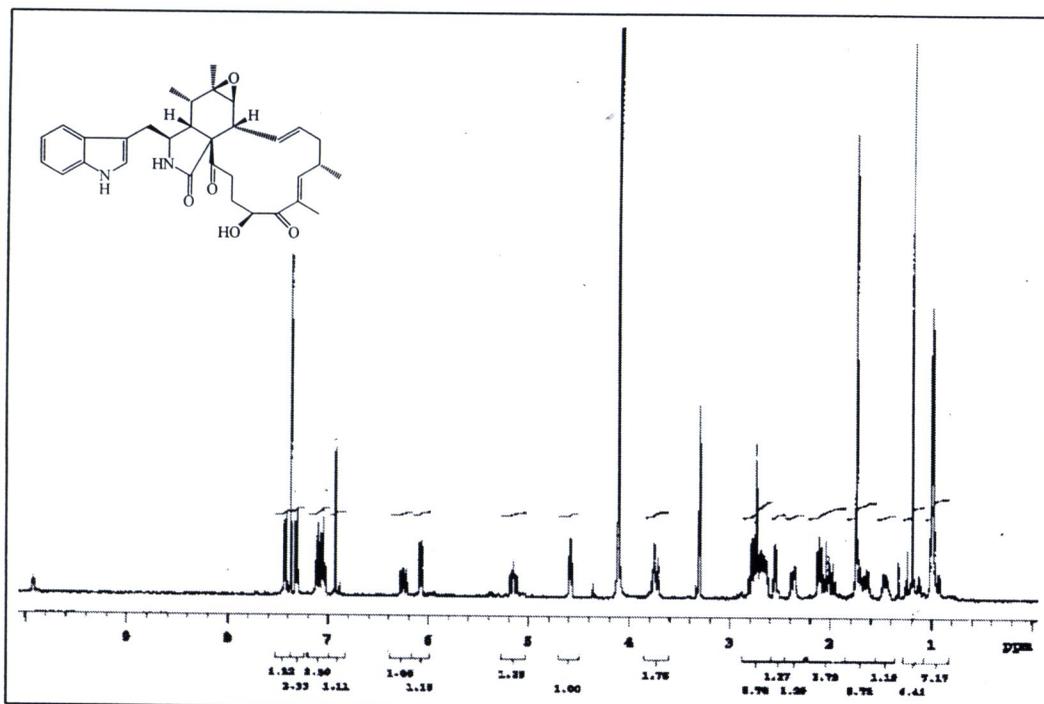


Figure 29 ^1H NMR spectrum of chaetoglobosin F (V).

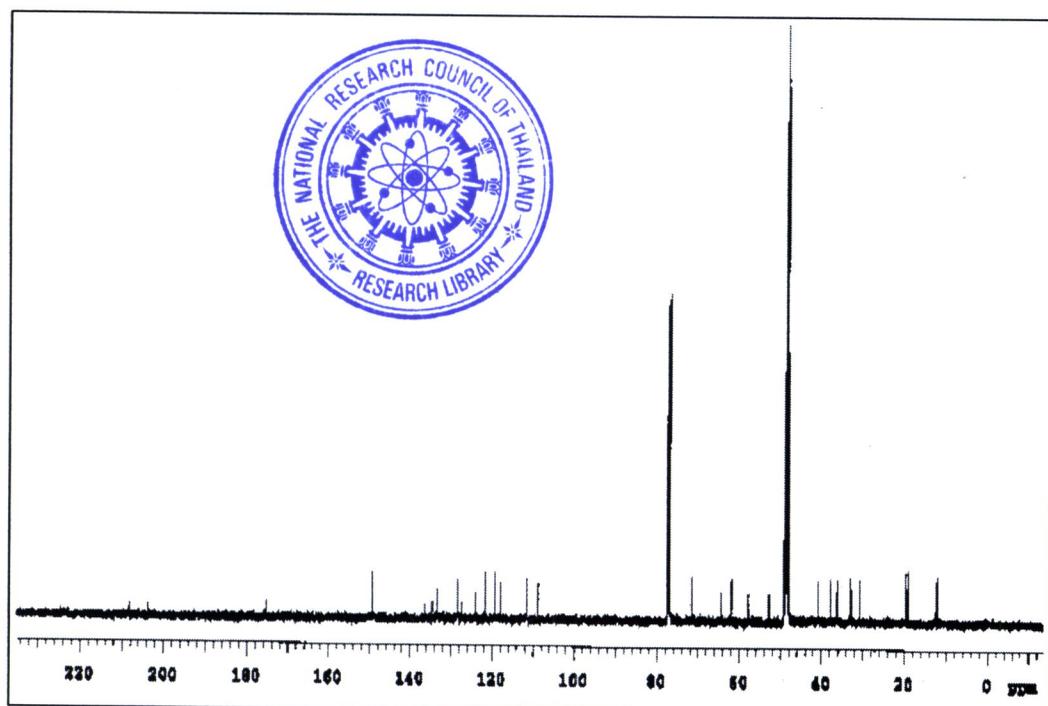


Figure 30 ^{13}C NMR spectrum of chaetoglobosin F (V).

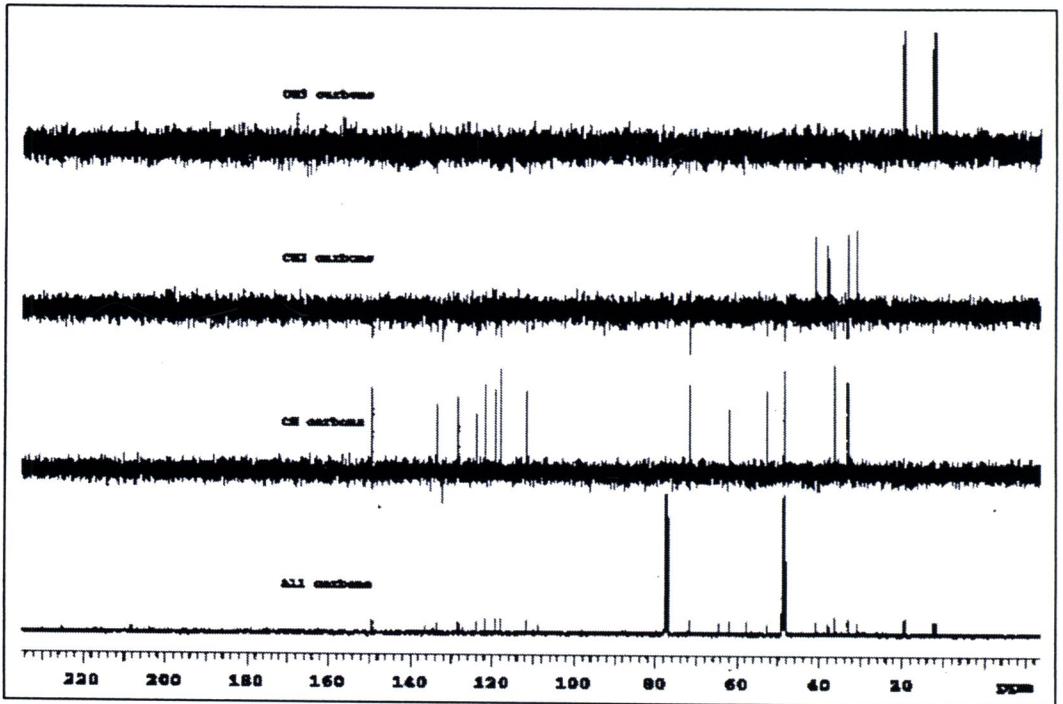


Figure 31 DEPT spectrum of chaetoglobosin F (V).

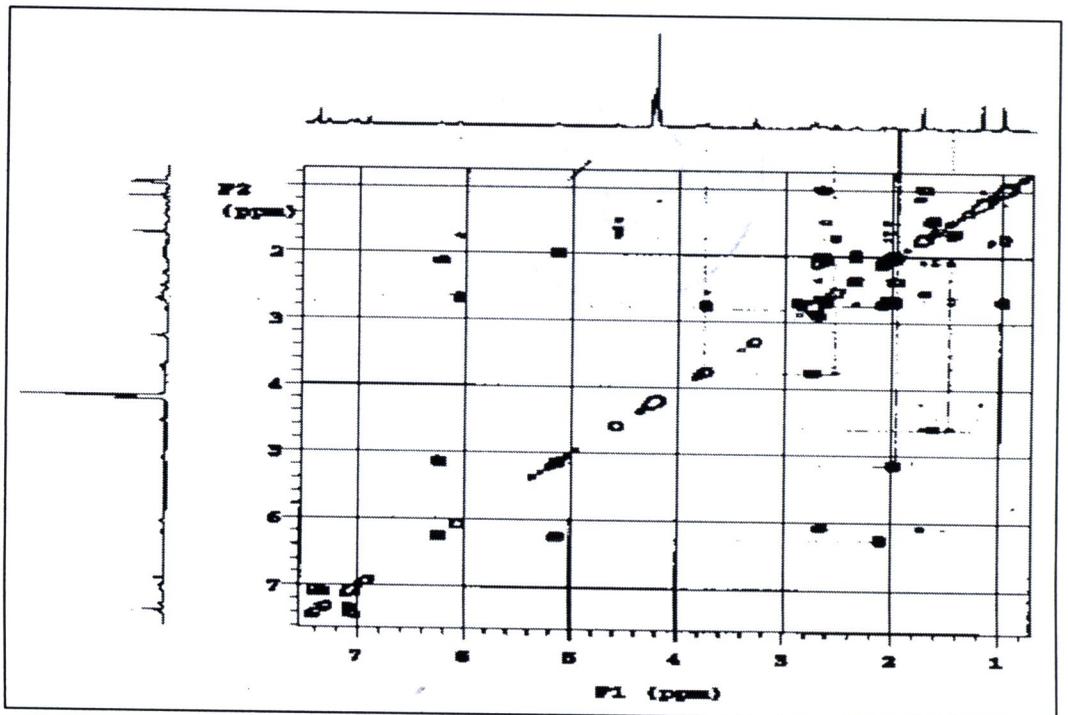


Figure 32 COSY spectrum of chaetoglobosin F (V).

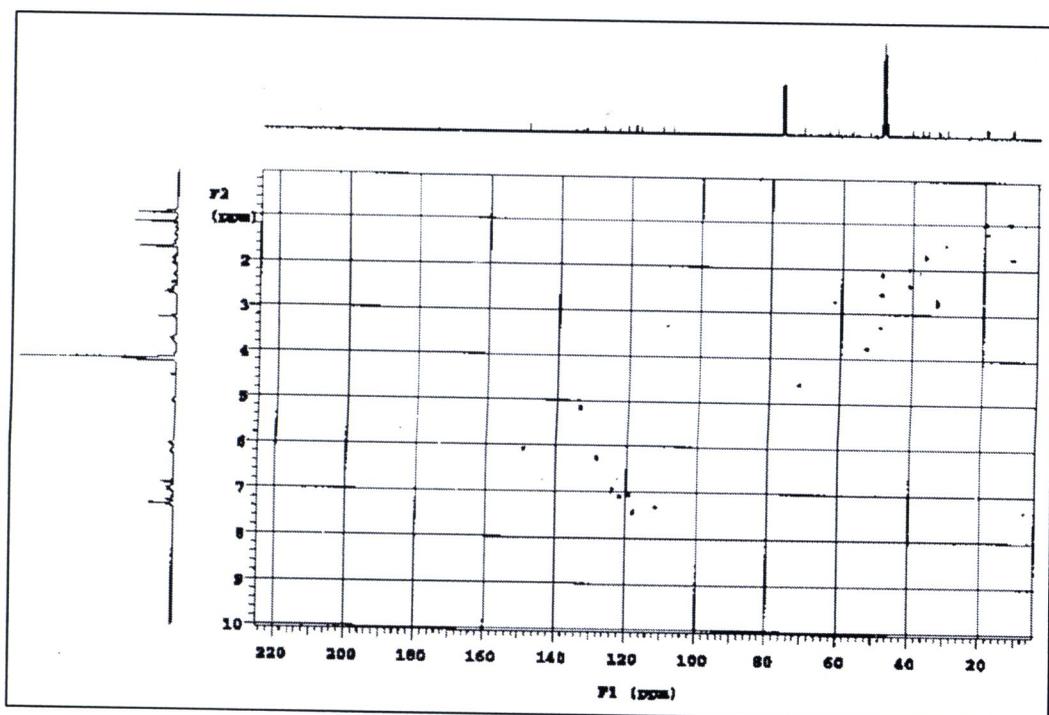


Figure 33 HSQC spectrum of chaetoglobosin F (V).

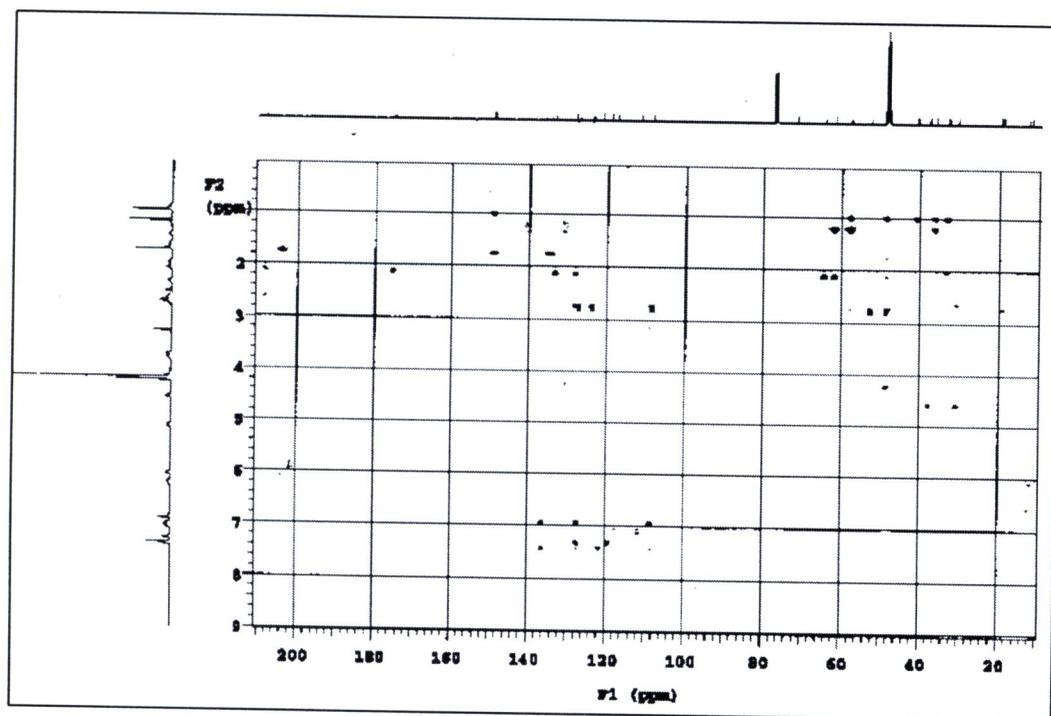


Figure 34 HMBC spectrum of chaetoglobosin F (V).

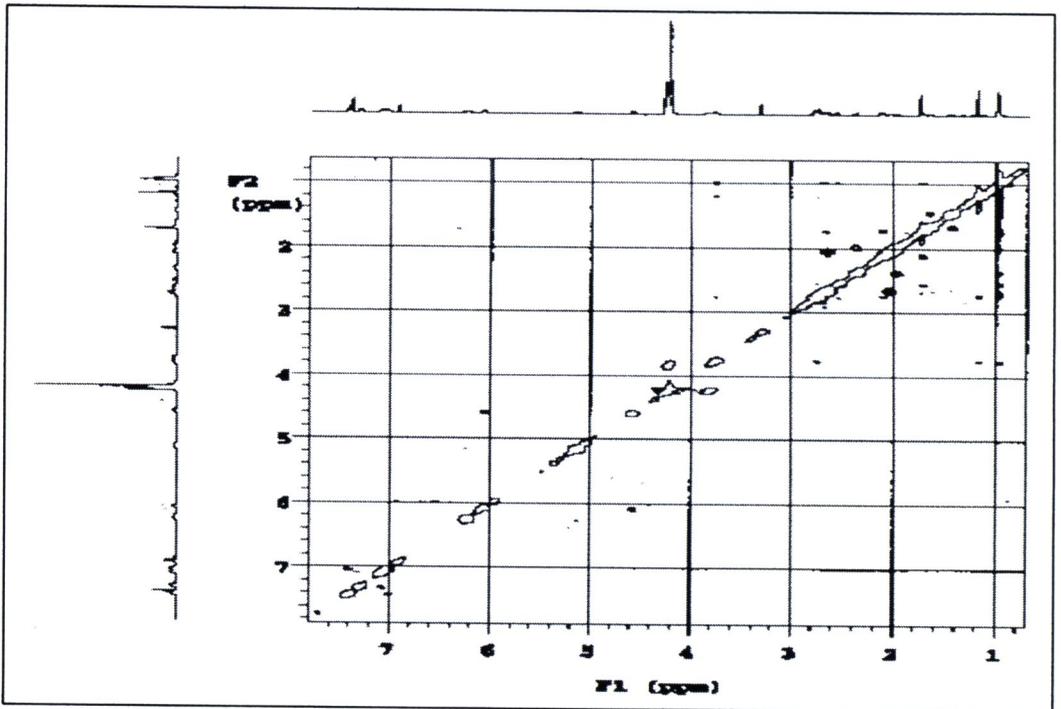


Figure 35 NOESY spectrum of chaetoglobosin F (V).

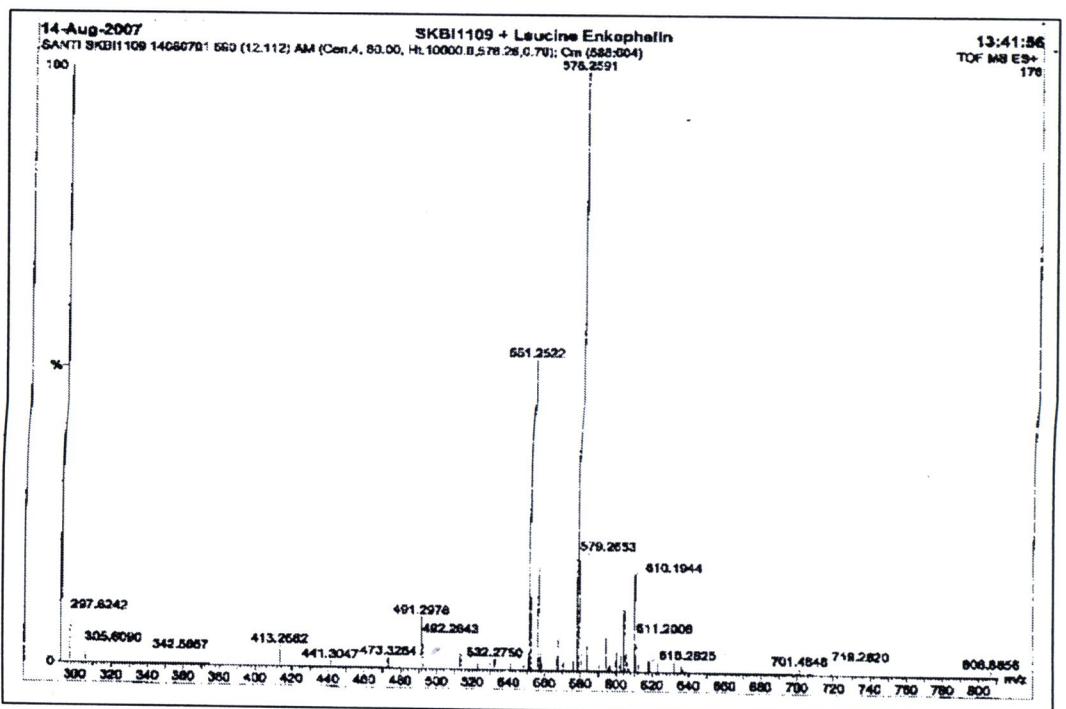


Figure 36 Mass spectrum of chaetoglobosin G (VI).

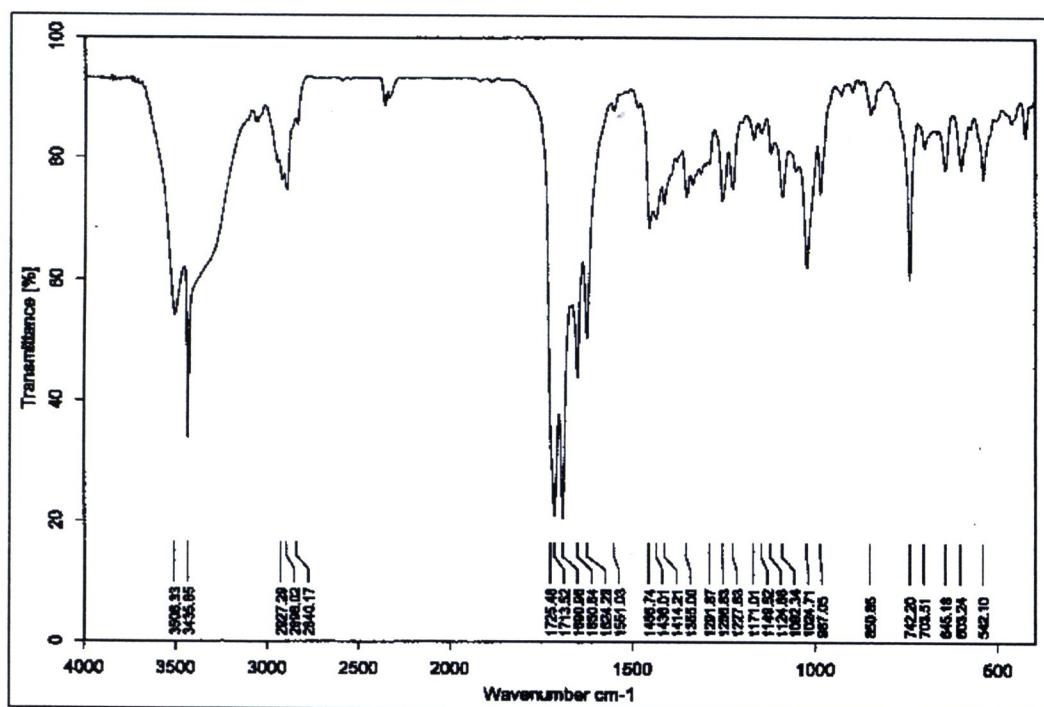


Figure 37 IR spectrum of chaetoglobosin G (VI).

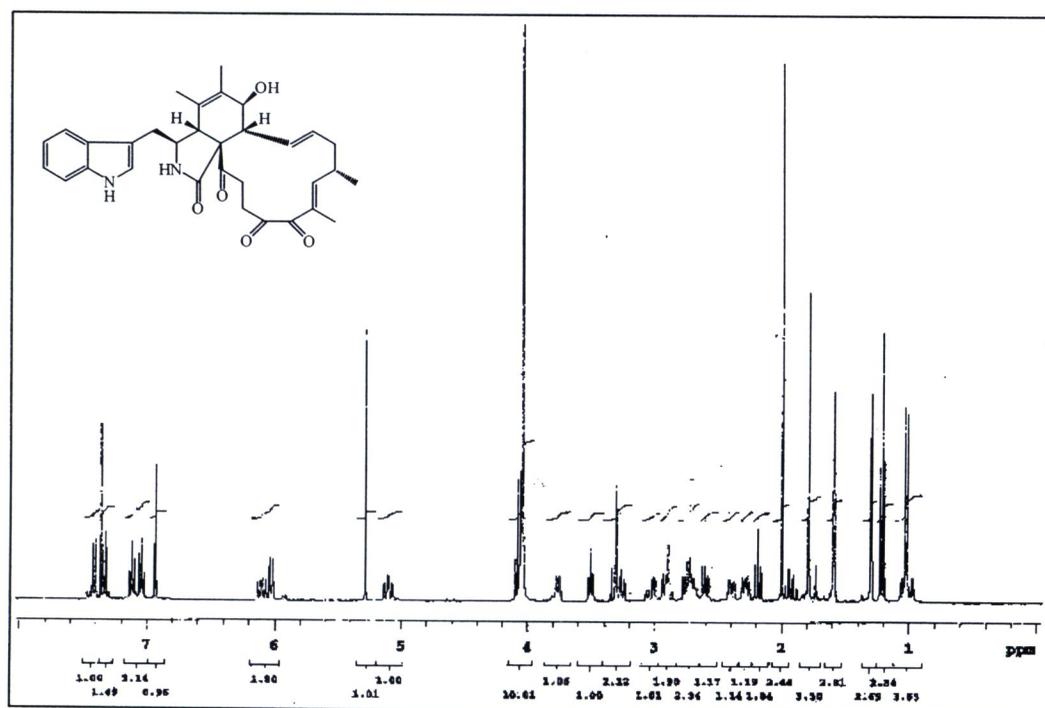


Figure 38 ^1H NMR spectrum of chaetoglobosin G (VI).

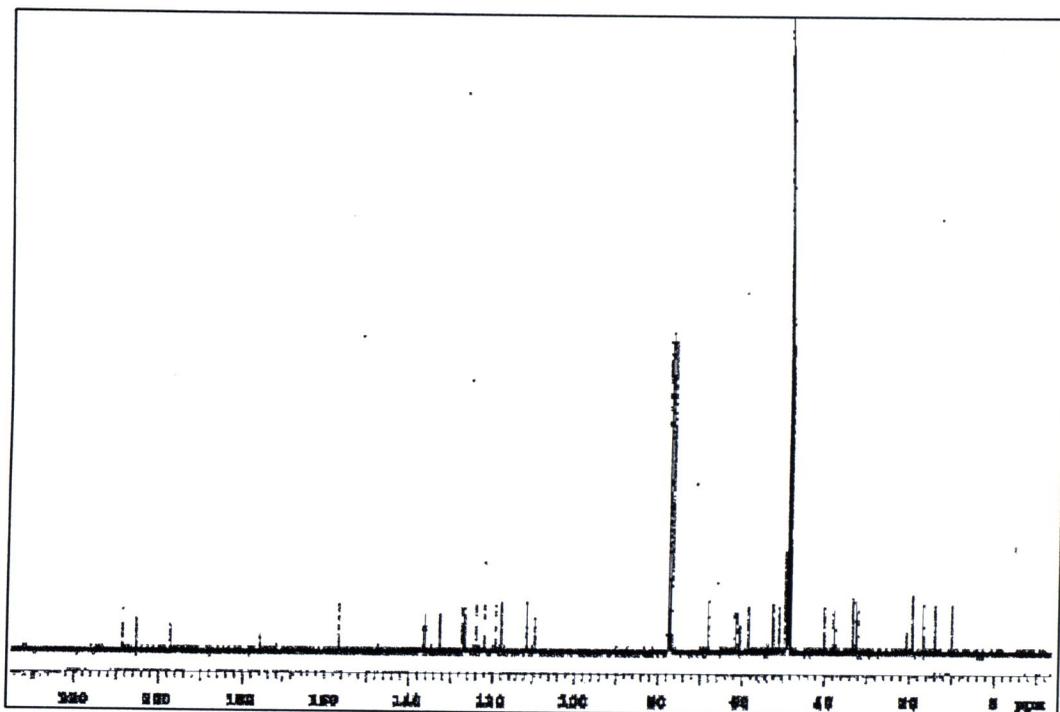


Figure 39 ^{13}C NMR spectrum of chaetoglobosin G (VI).

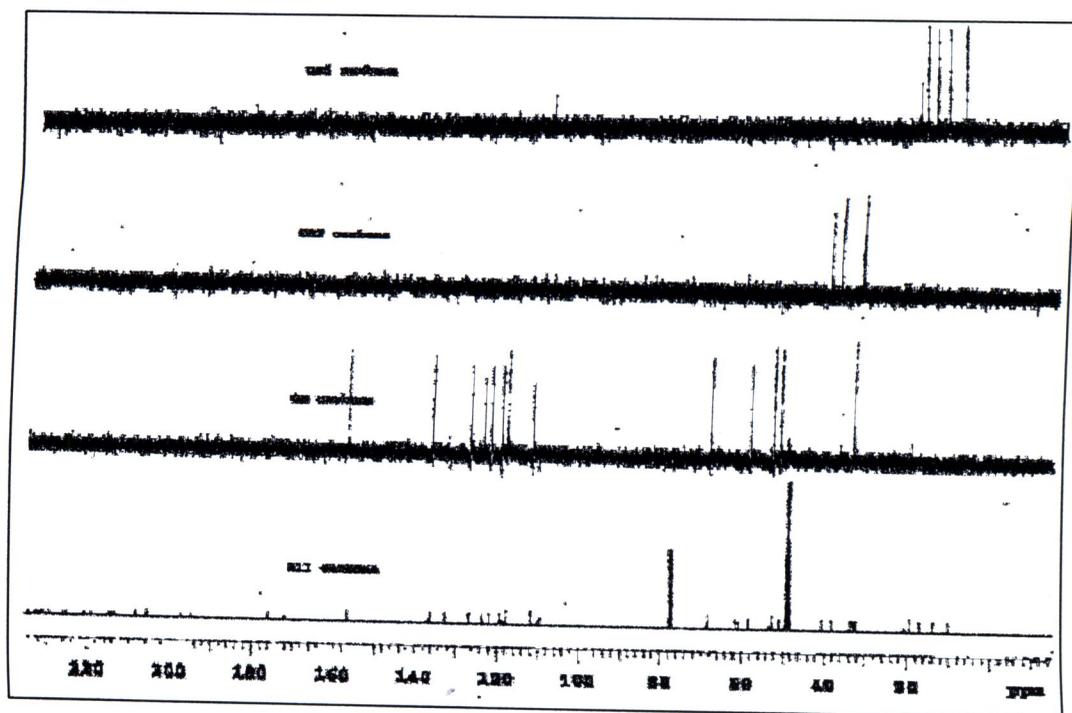


Figure 40 DEPT spectrum of chaetoglobosin G (VI).

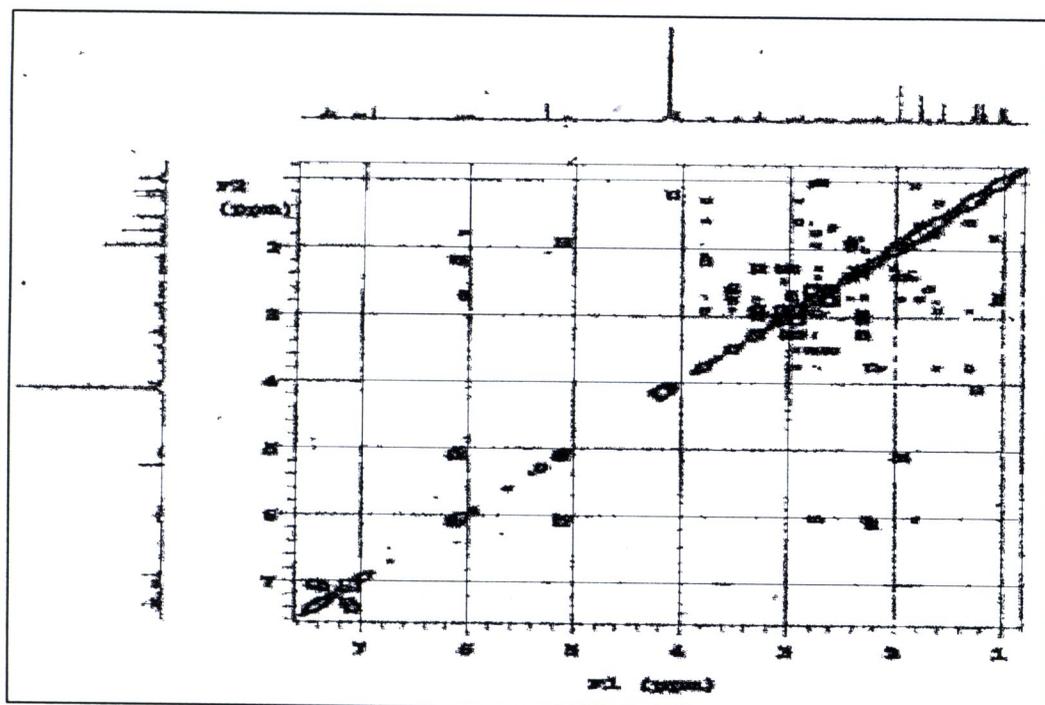


Figure 41 COSY spectrum of chaetoglobosin G (VI).

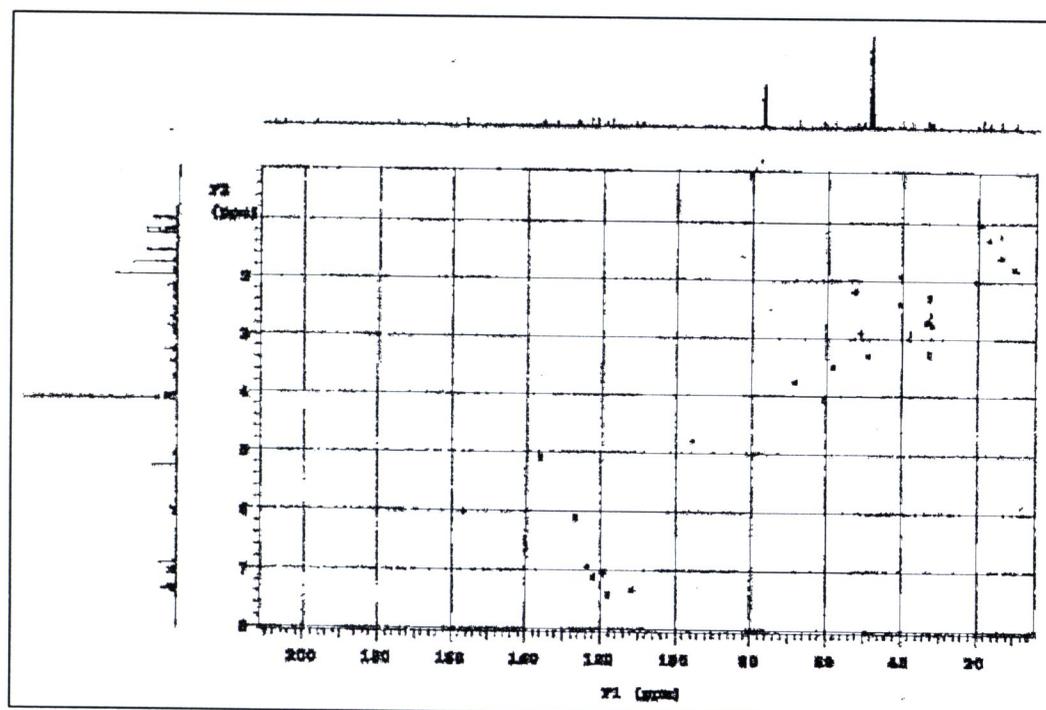


Figure 42 HSQC spectrum of chaetoglobosin G (VI).

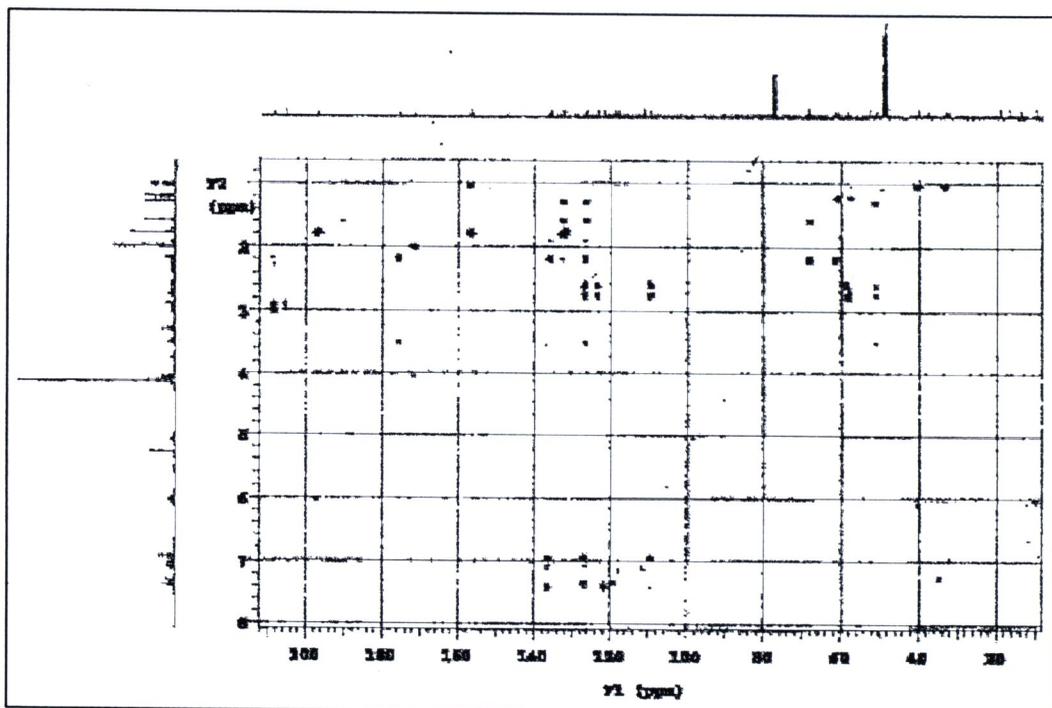


Figure 43 HMBC spectrum of chaetoglobosin G (VI).

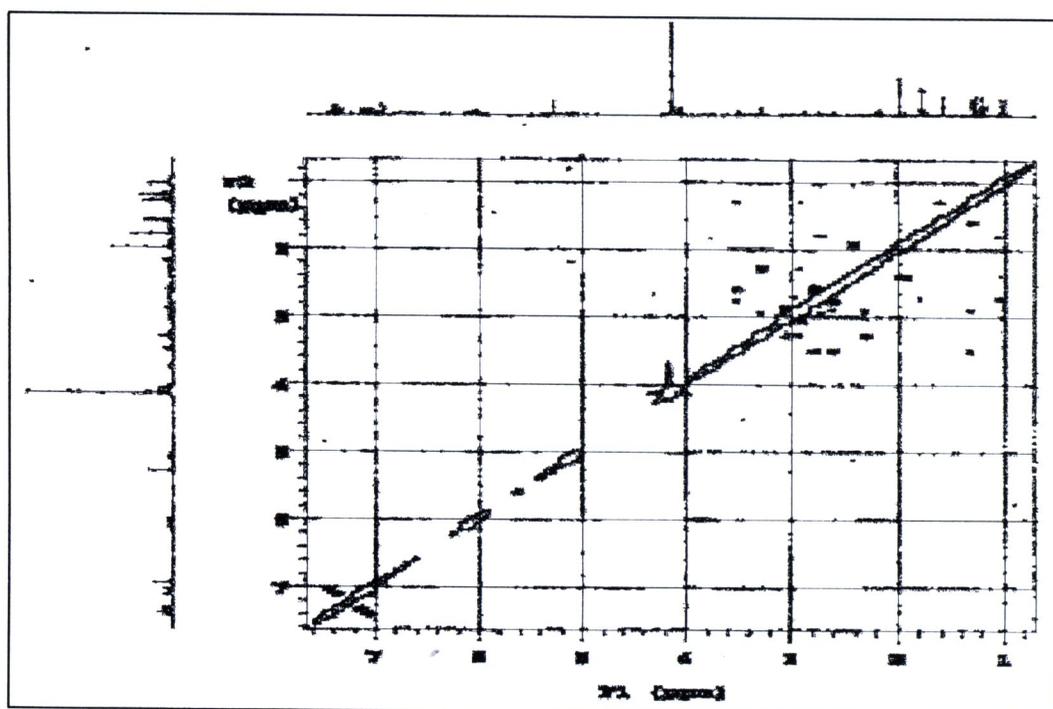


Figure 44 NOESY spectrum of chaetoglobosin G (VI).

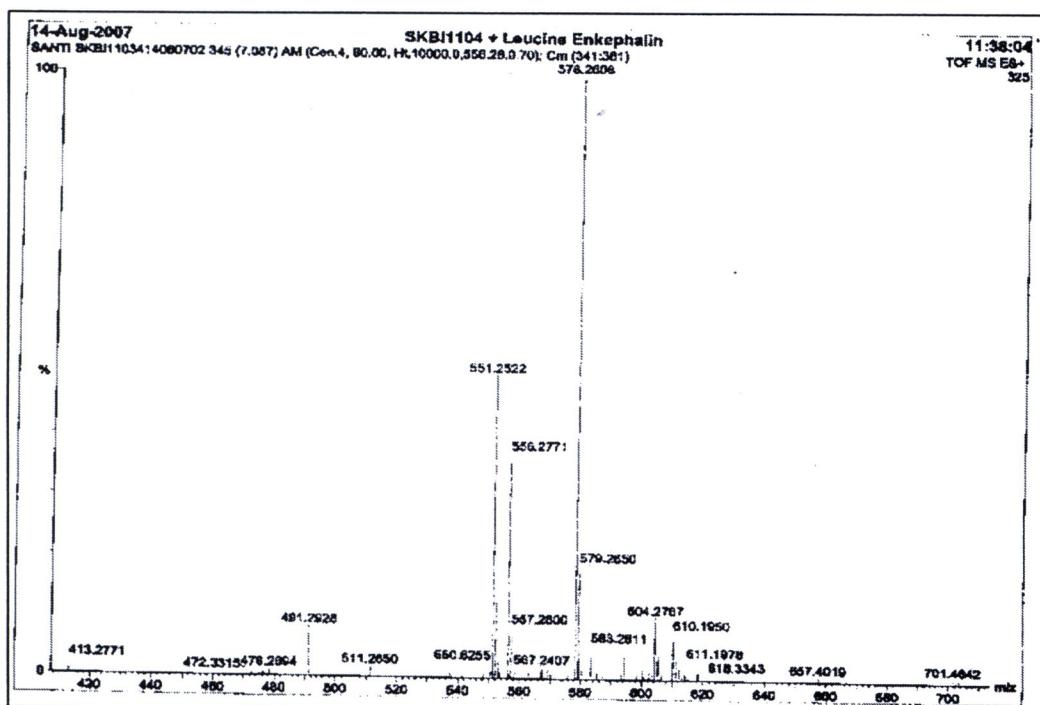


Figure 45 Mass spectrum of chaetoglobosin B (VII).

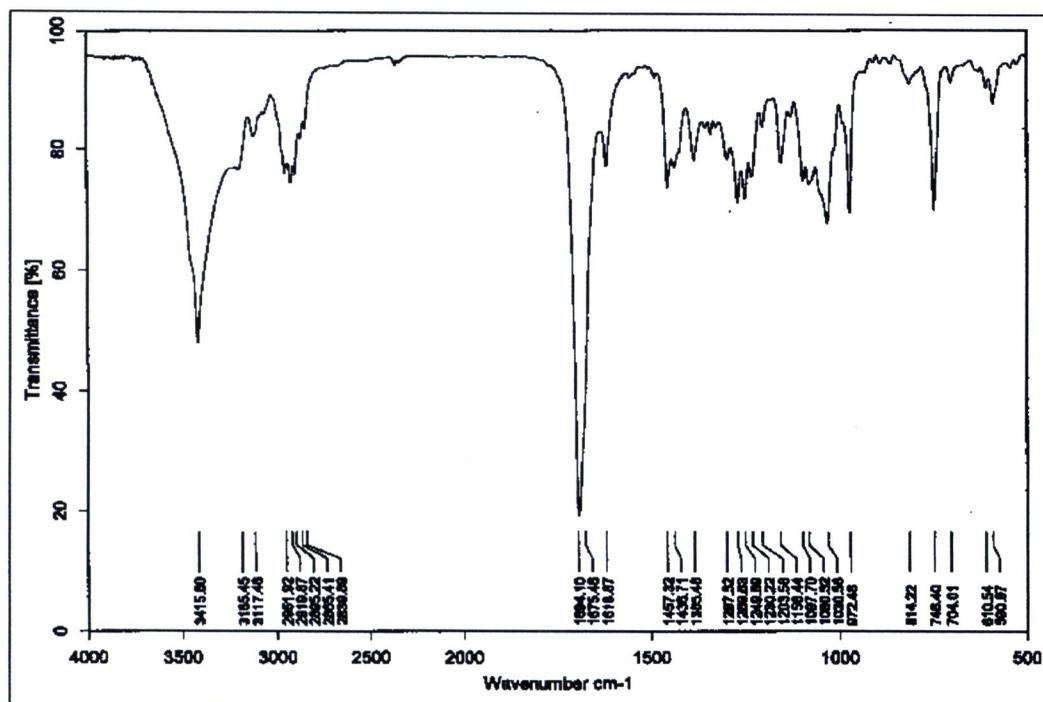


Figure 46 IR spectrum of chaetoglobosin B (VII).

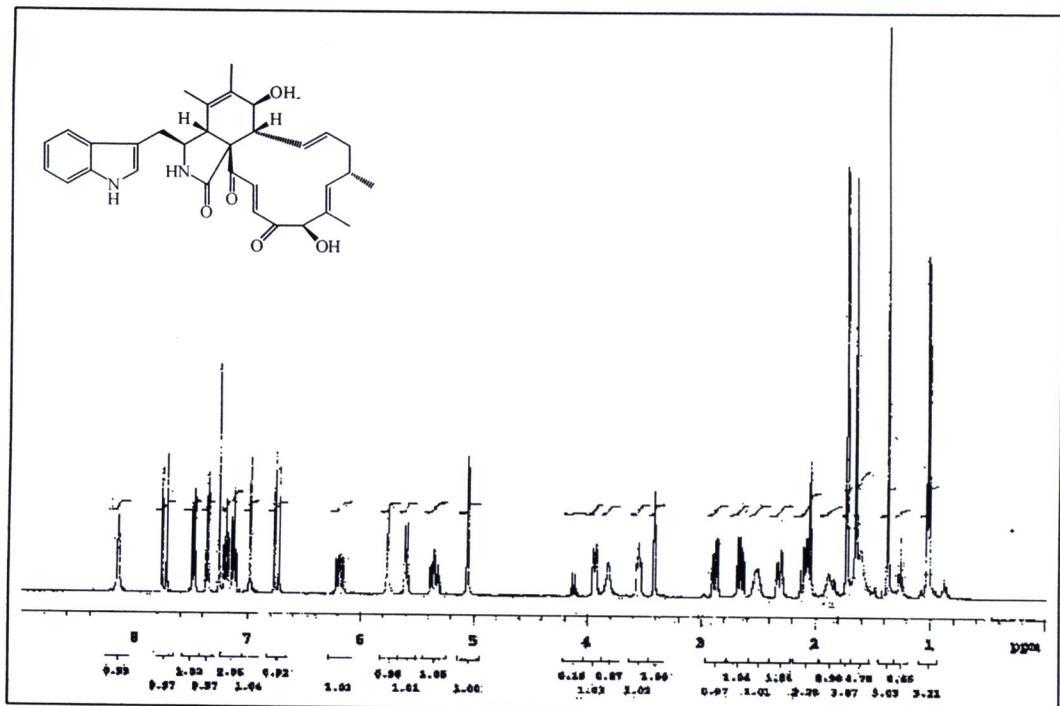


Figure 47 ^1H NMR spectrum of chaetoglobosin B (VI).

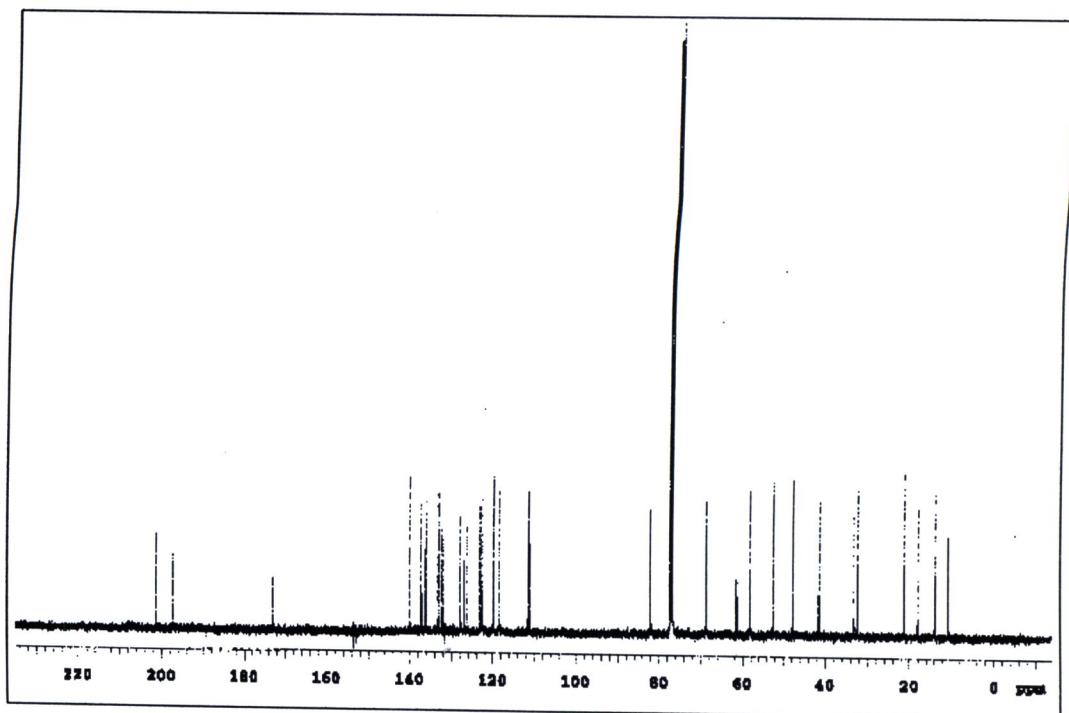


Figure 48 ^{13}C NMR spectrum of chaetoglobosin B (VII).

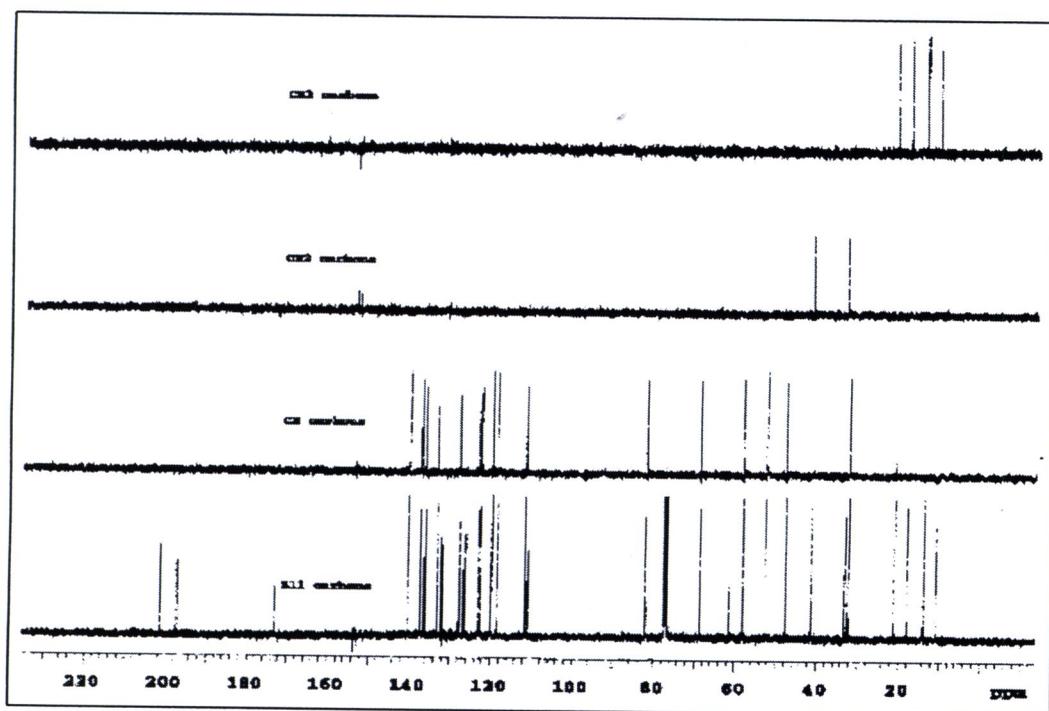


Figure 49 DEPT spectrum of chaetoglobosin B (VII).

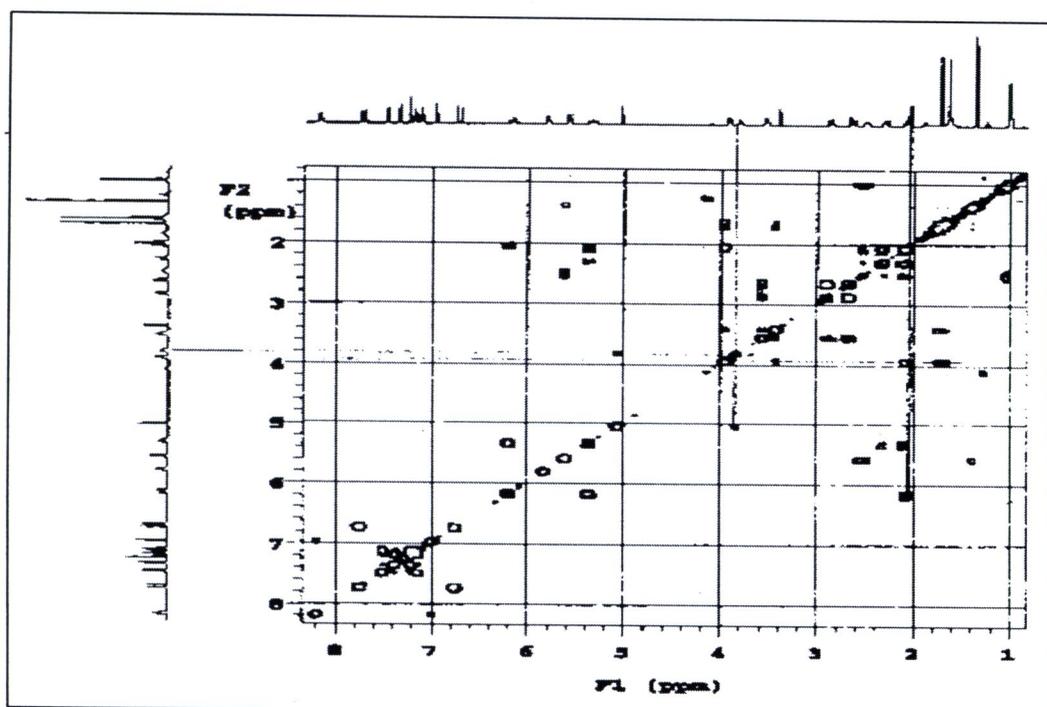


Figure 50 COSY spectrum of chaetoglobosin B (VII).

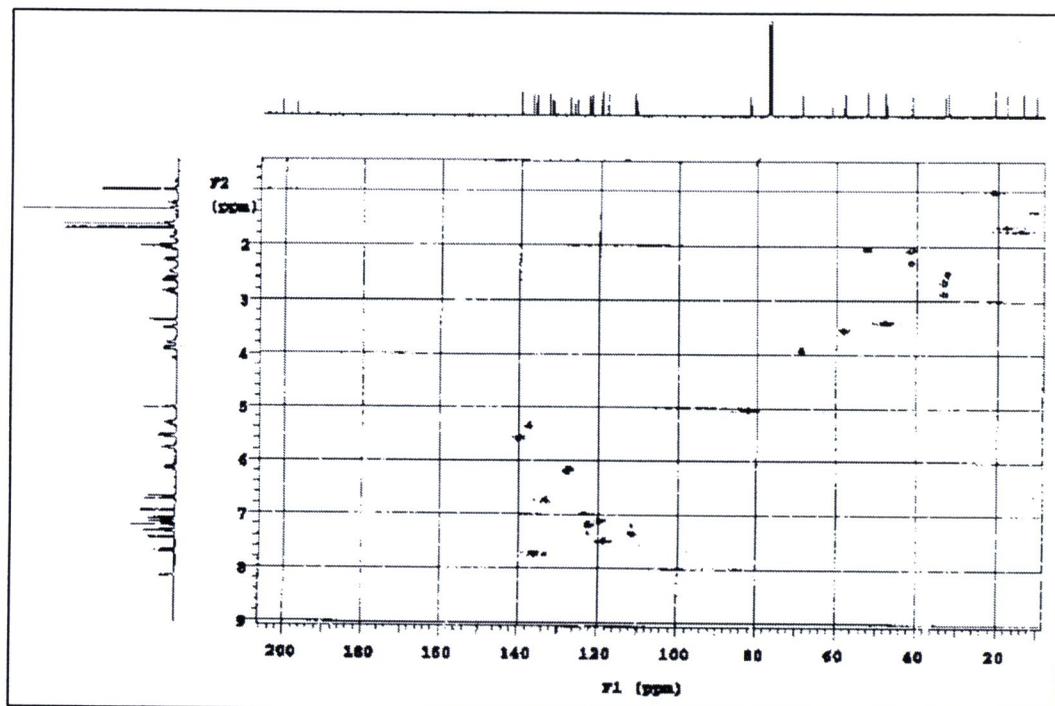


Figure 51 HSQC spectrum of chaetoglobosin B (VII).

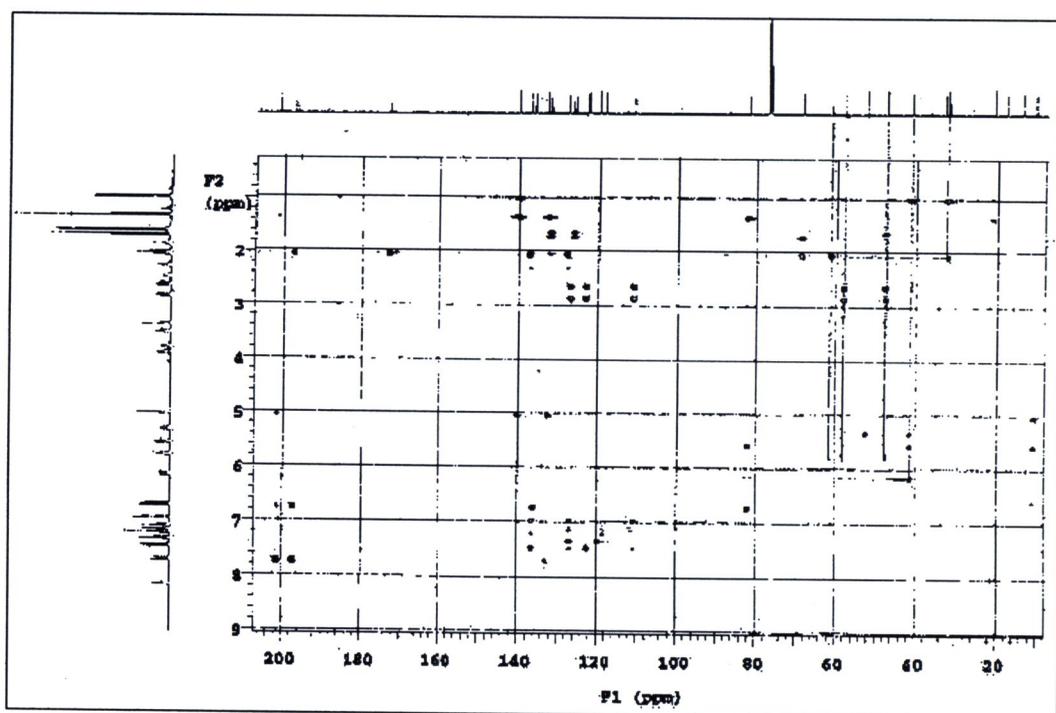


Figure 52 HMBC spectrum of chaetoglobosin B (VII).

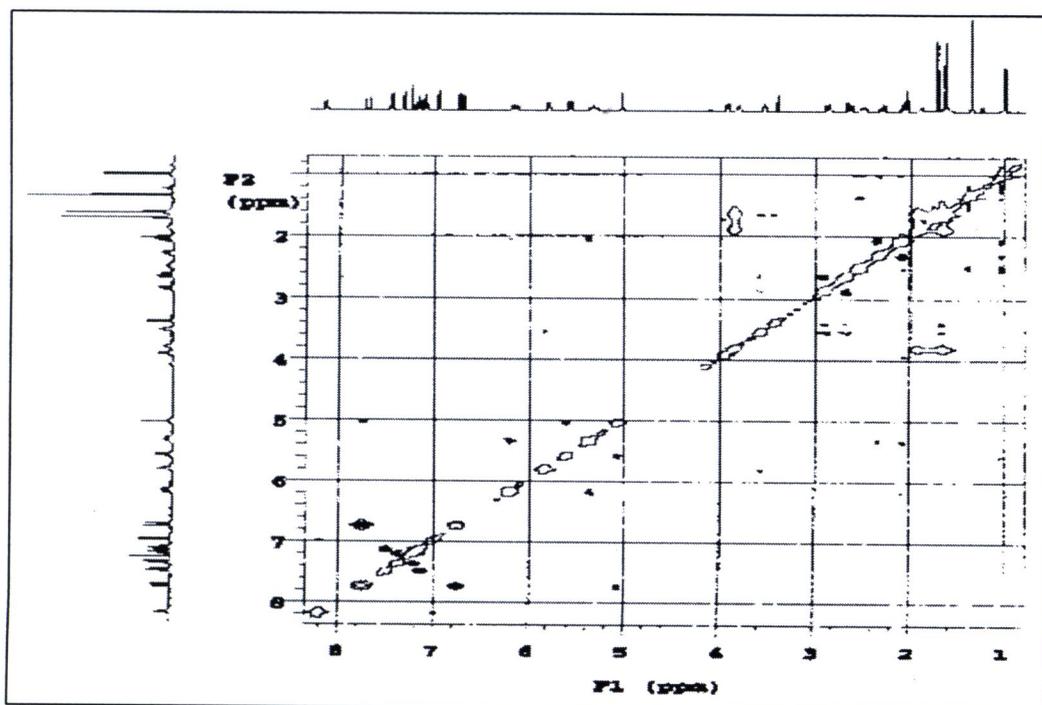


Figure 53 NOESY spectrum of chaetoglobosin B (VII).

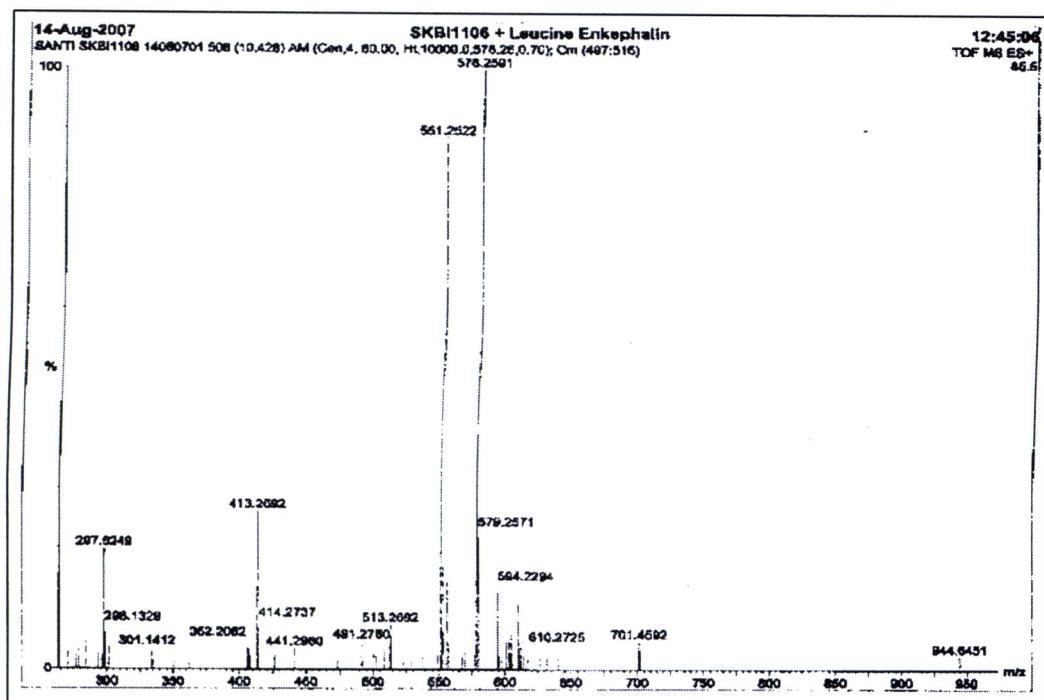


Figure 54 Mass spectrum of chaetoglobosin D (VIII).

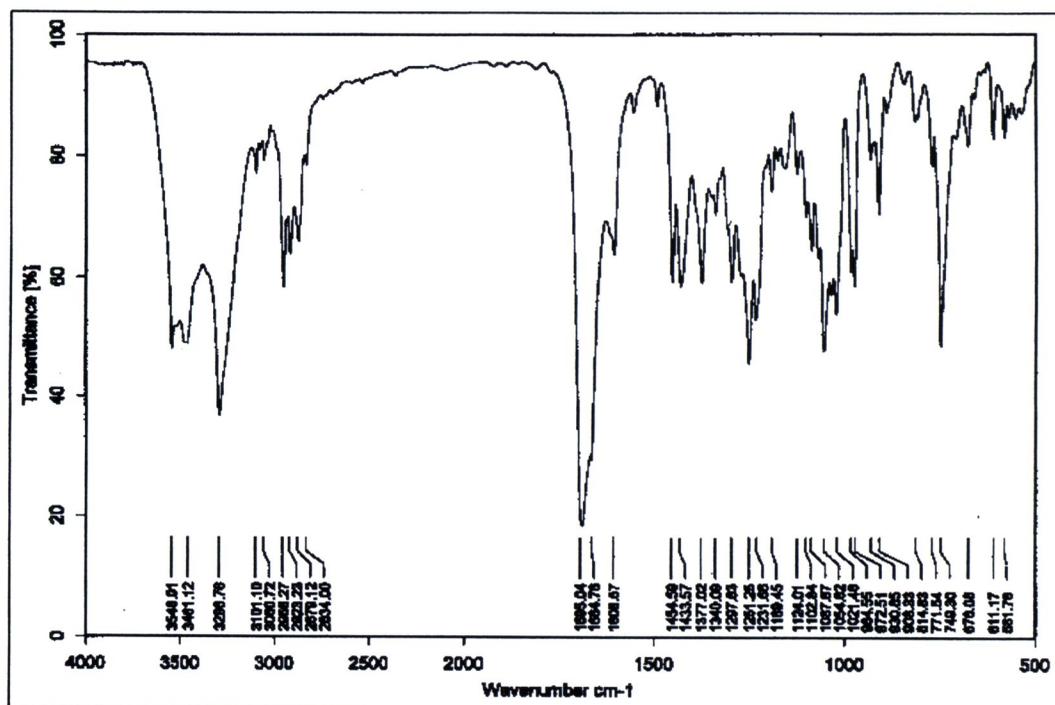


Figure 55 IR spectrum of chaetoglobosin D (VIII).

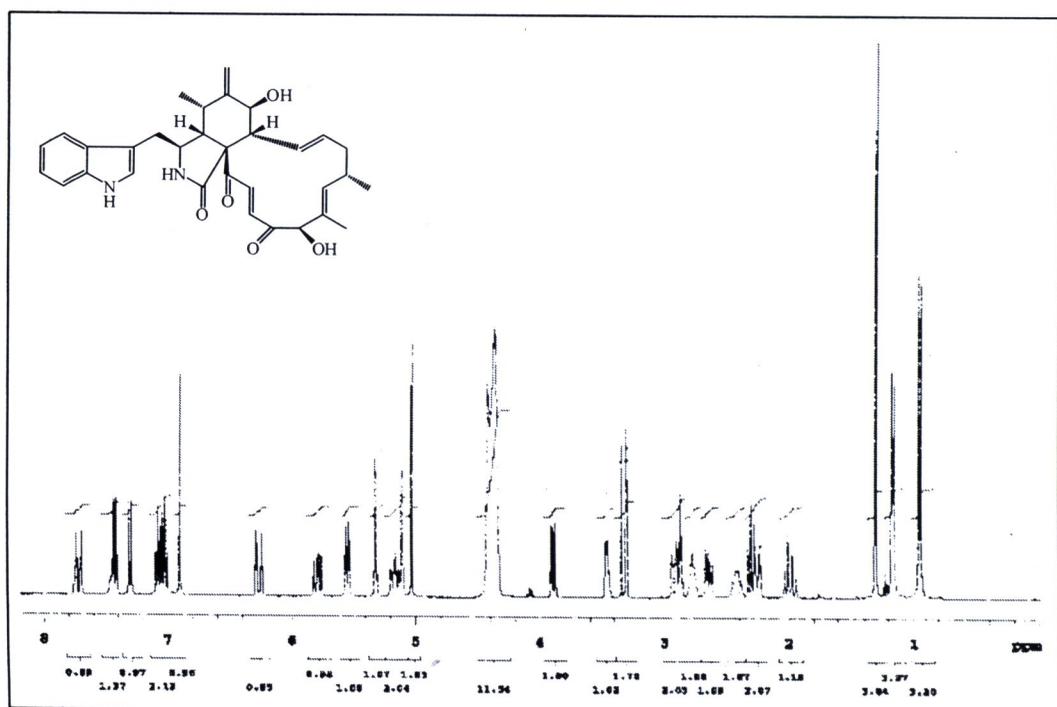


Figure 56 ¹H NMR spectrum of chaetoglobosin D (VIII).

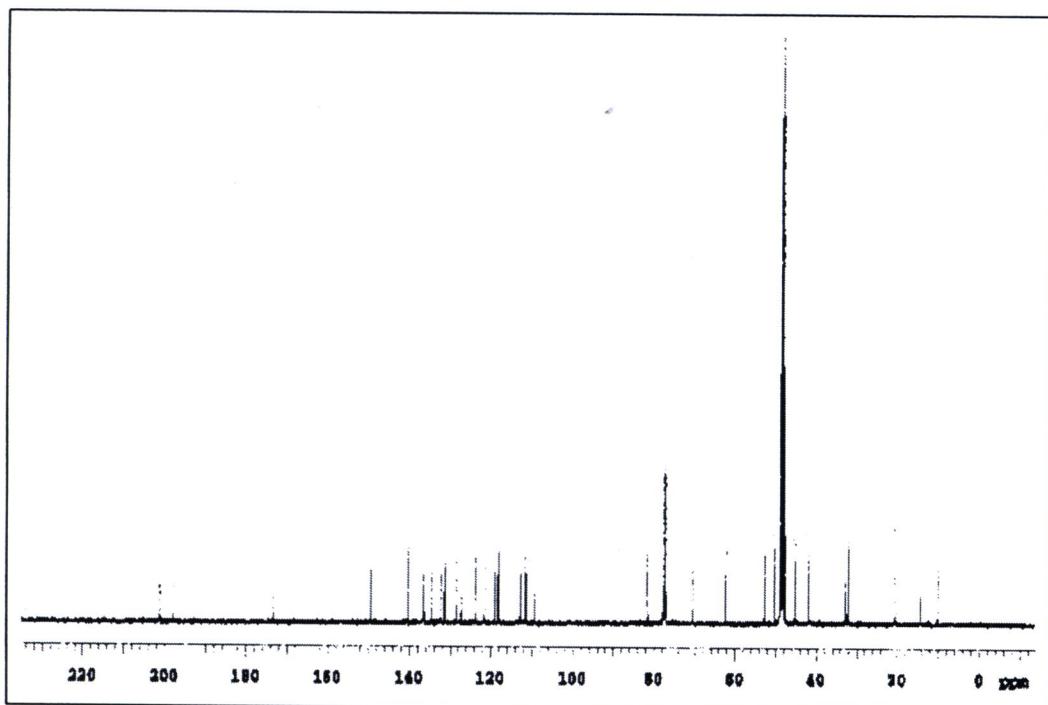


Figure 57 ^{13}C NMR spectrum of chaetoglobosin D (VIII).

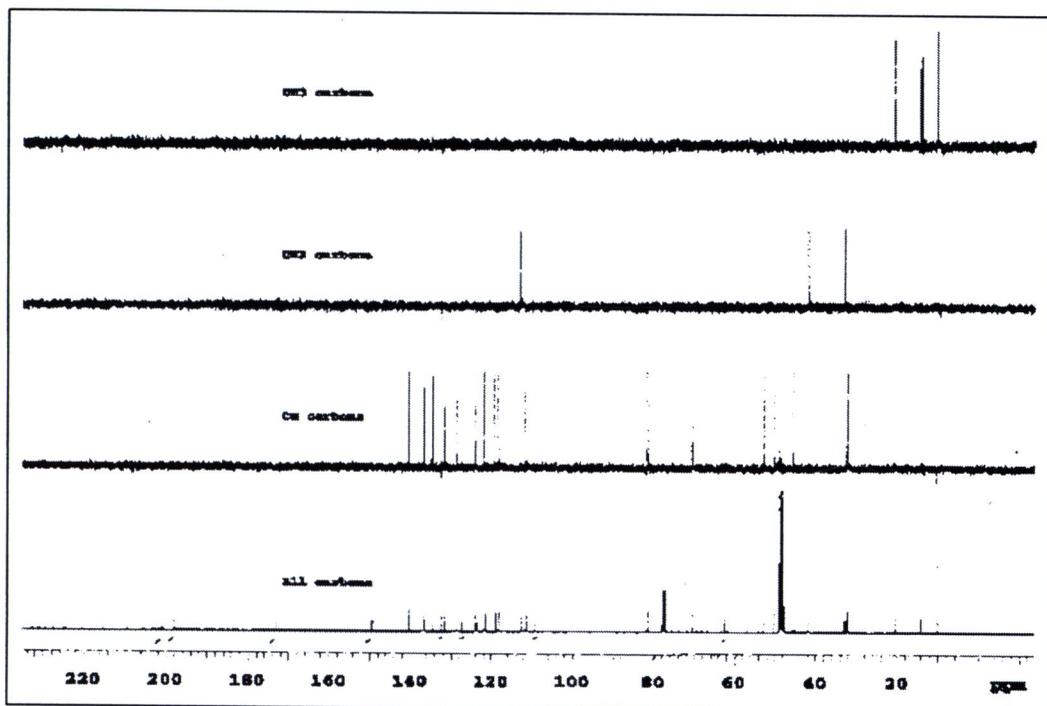


Figure 58 DEPT spectrum of chaetoglobosin D (VIII).

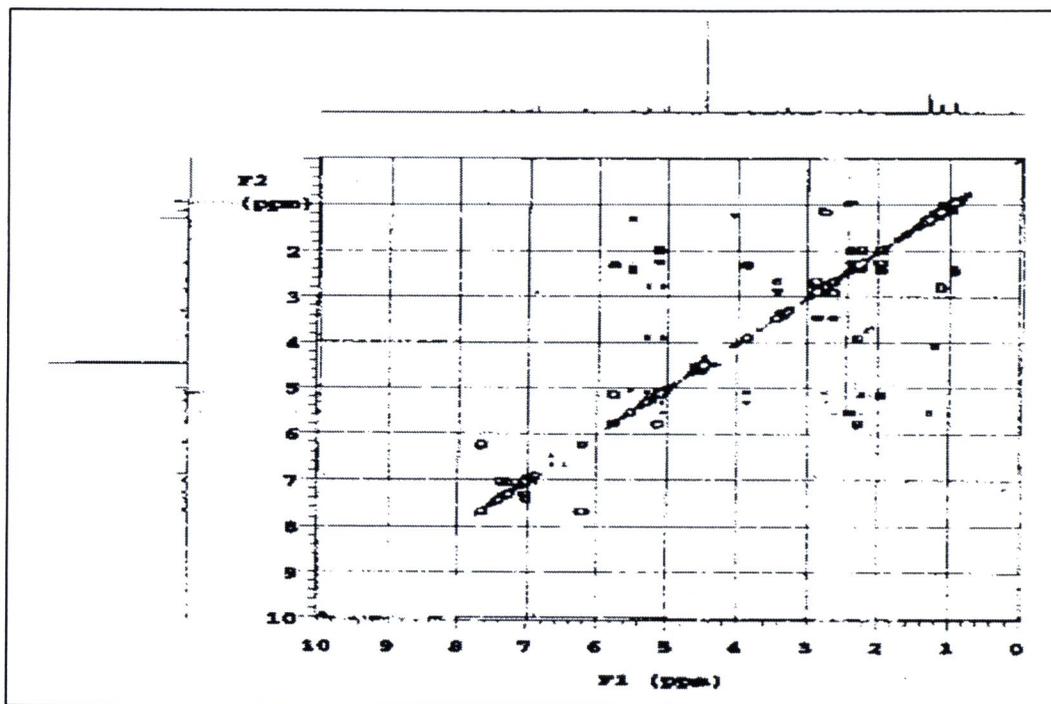


Figure 59 COSY spectrum of chaetoglobosin D (VIII).

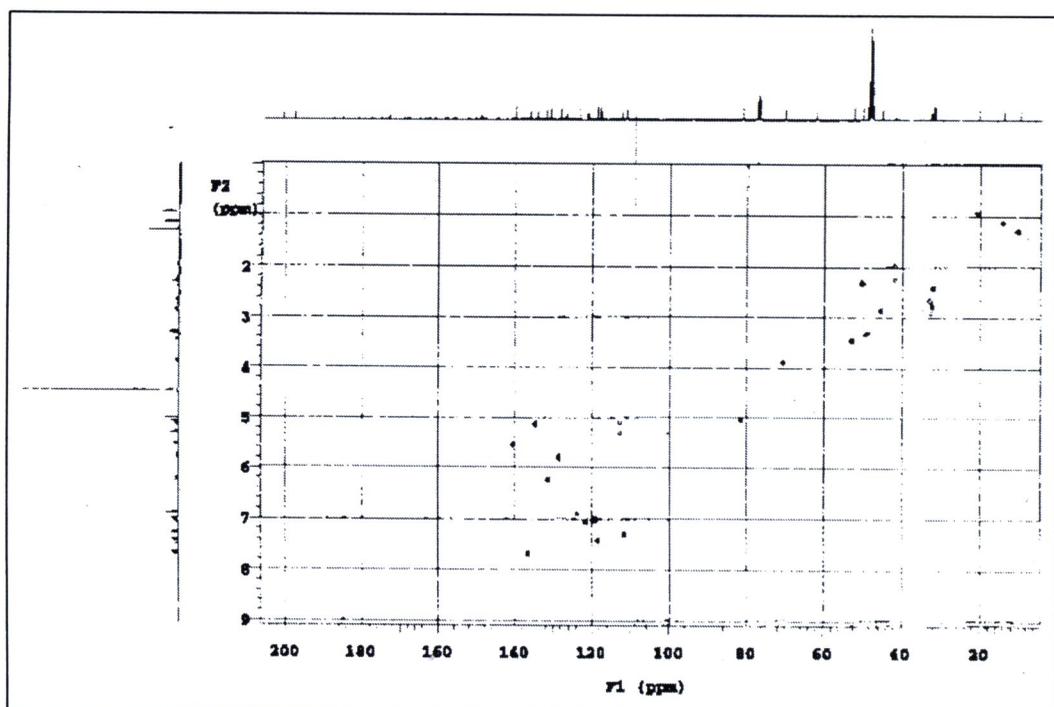


Figure 60 HSQC spectrum of chaetoglobosin D (VIII).

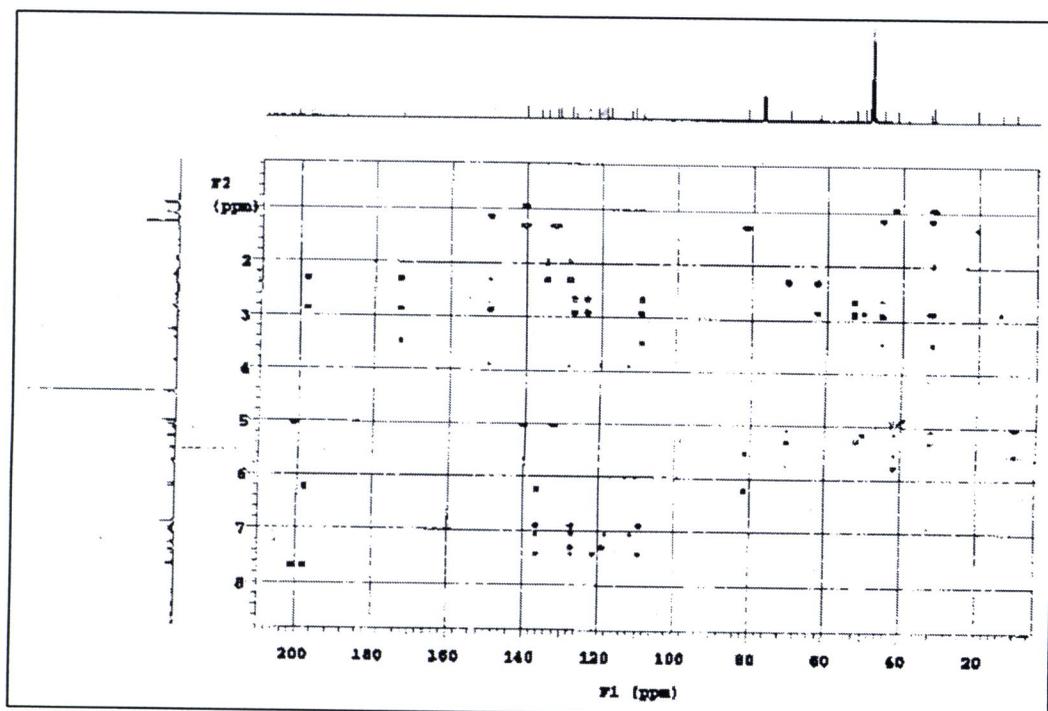


Figure 61 HMBC spectrum of chaetoglobosin D (VIII).

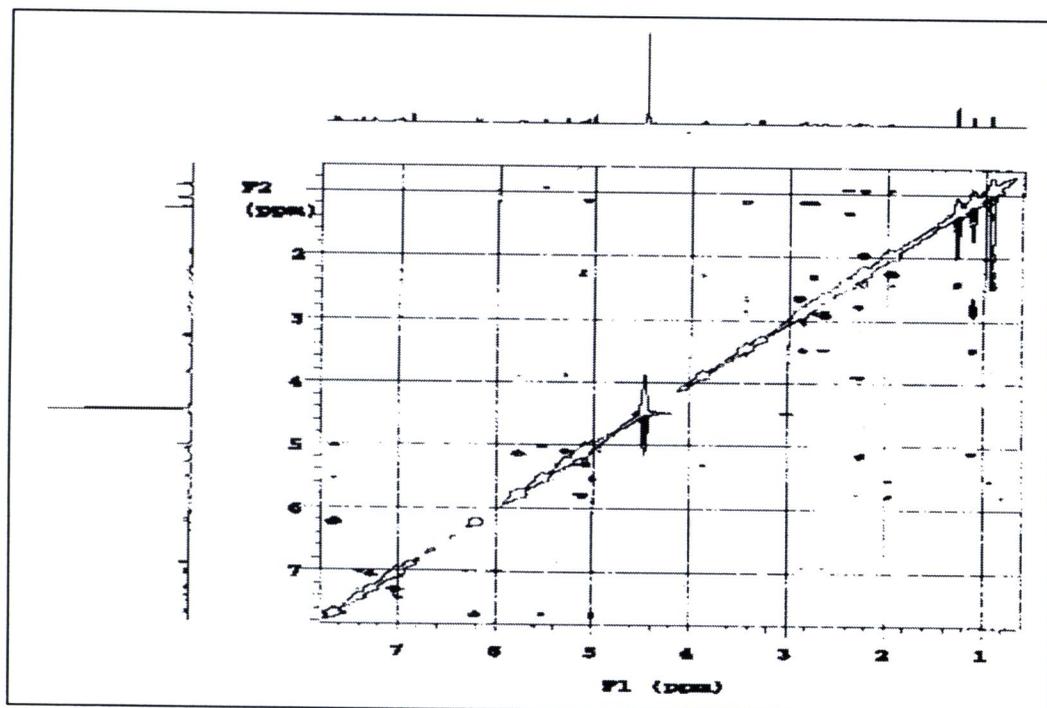


Figure 62 NOESY spectrum of chaetoglobosin D (VIII).

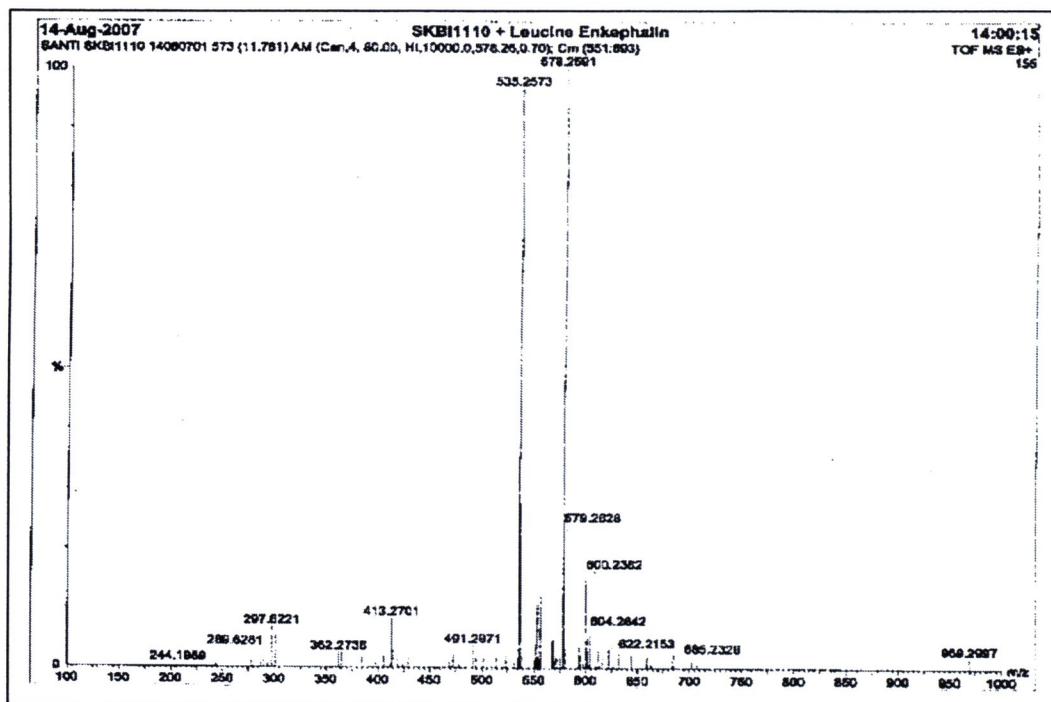


Figure 63 Mass spectrum of prochaetoglobosin III (IX).

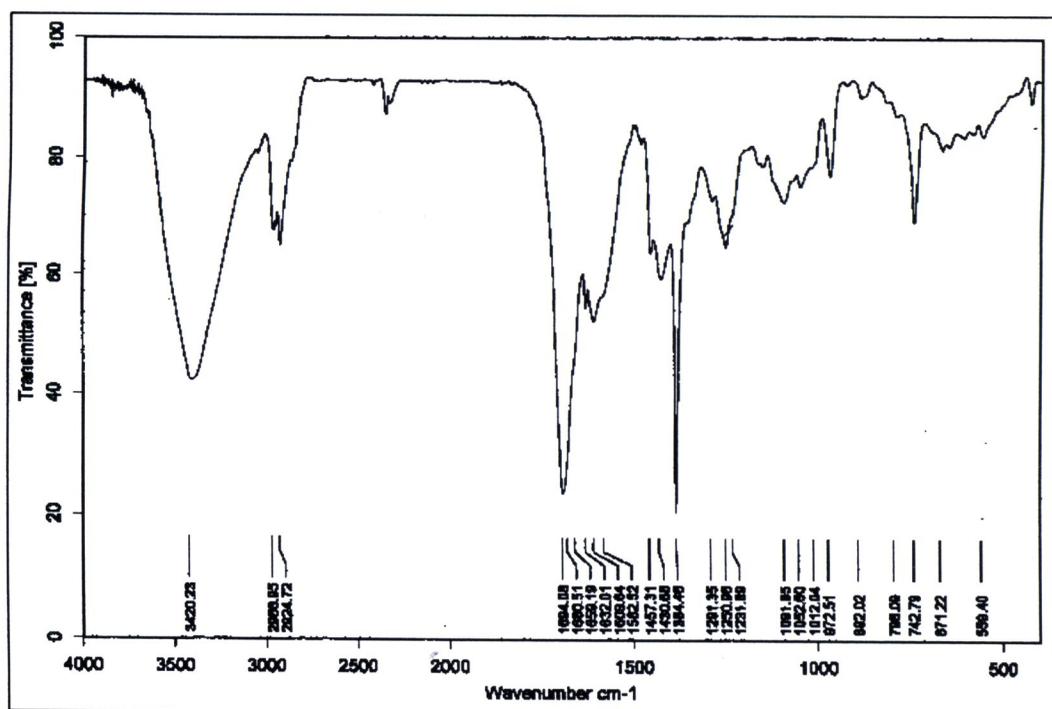


Figure 64 IR spectrum of prochaetoglobosin III (IX).

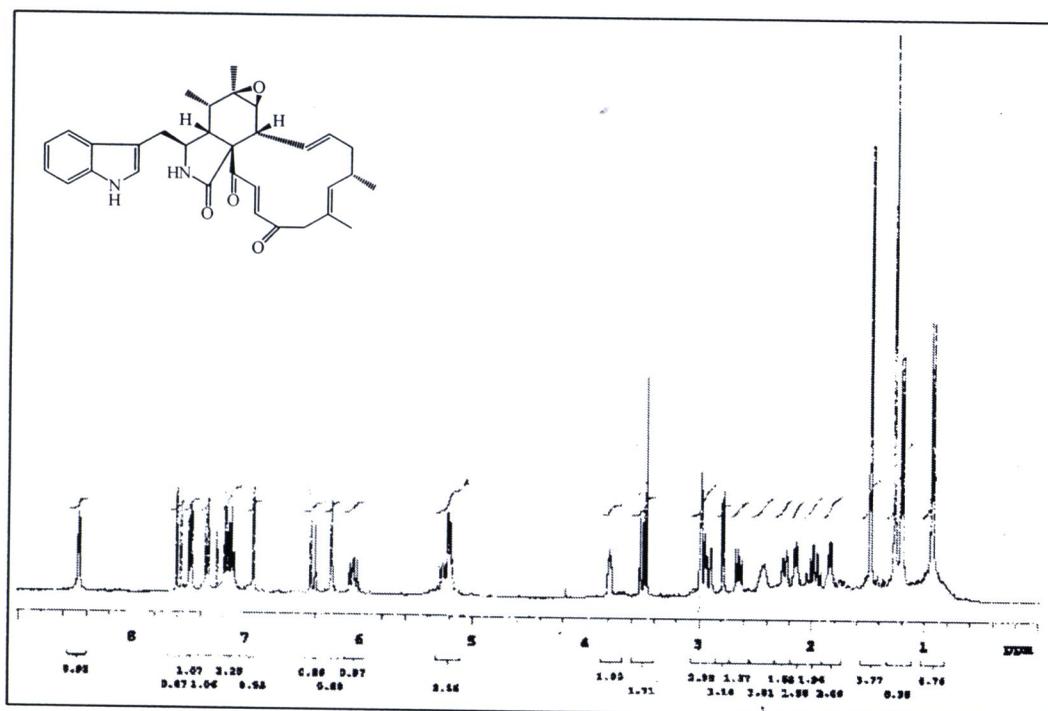


Figure 65 ^1H NMR spectrum of prochaetoglobosin III (IX).

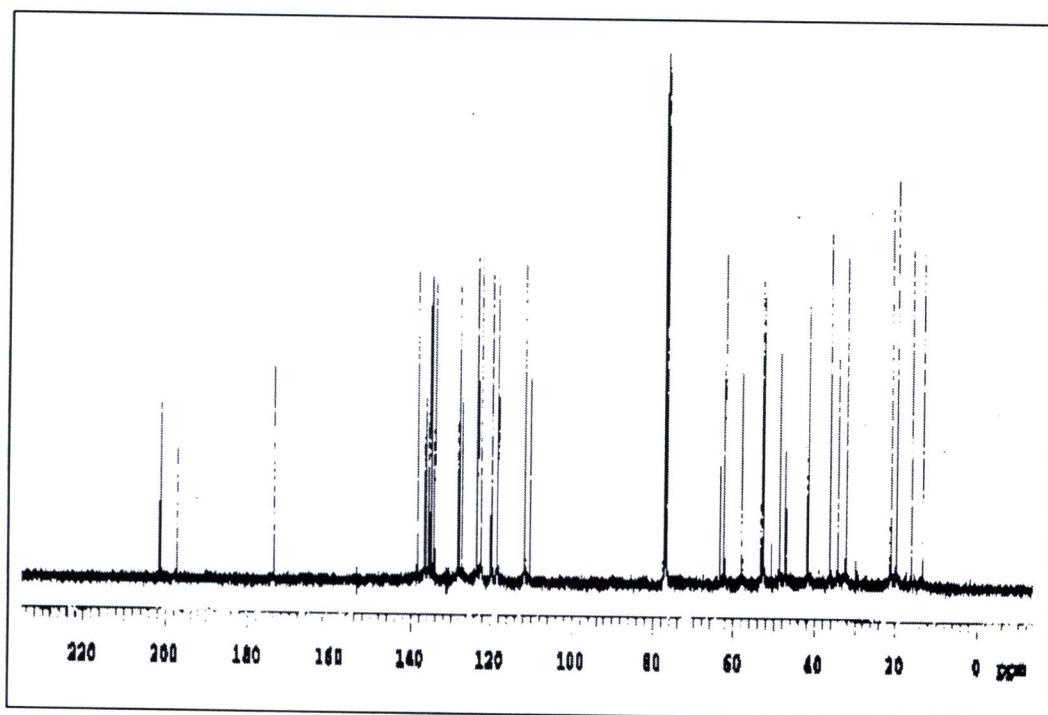


Figure 66 ^{13}C NMR spectrum of prochaetoglobosin III (IX).

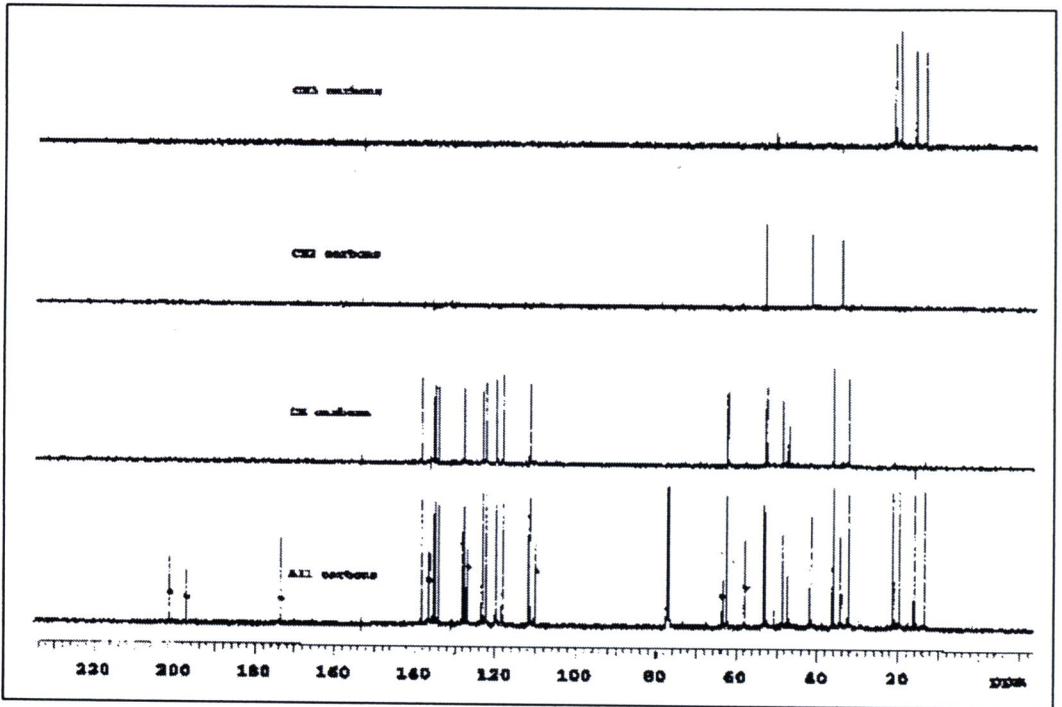


Figure 67 DEPT spectrum of prochaetoglobosin III (IX).

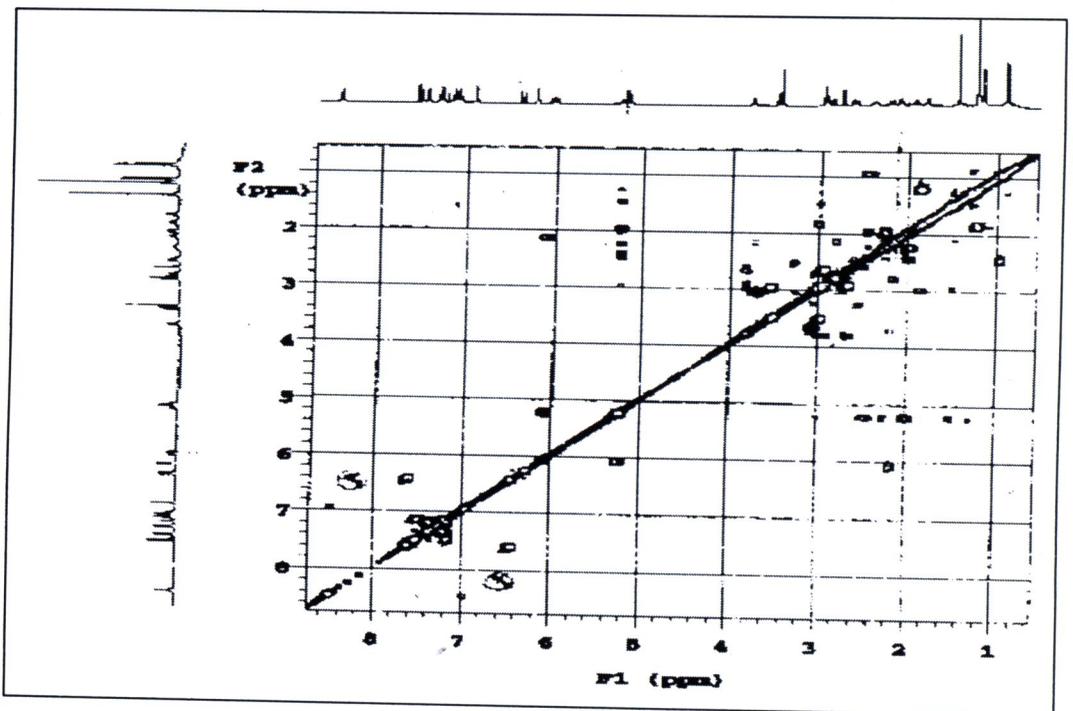


Figure 68 COSY spectrum of prochaetoglobosin III (IX).

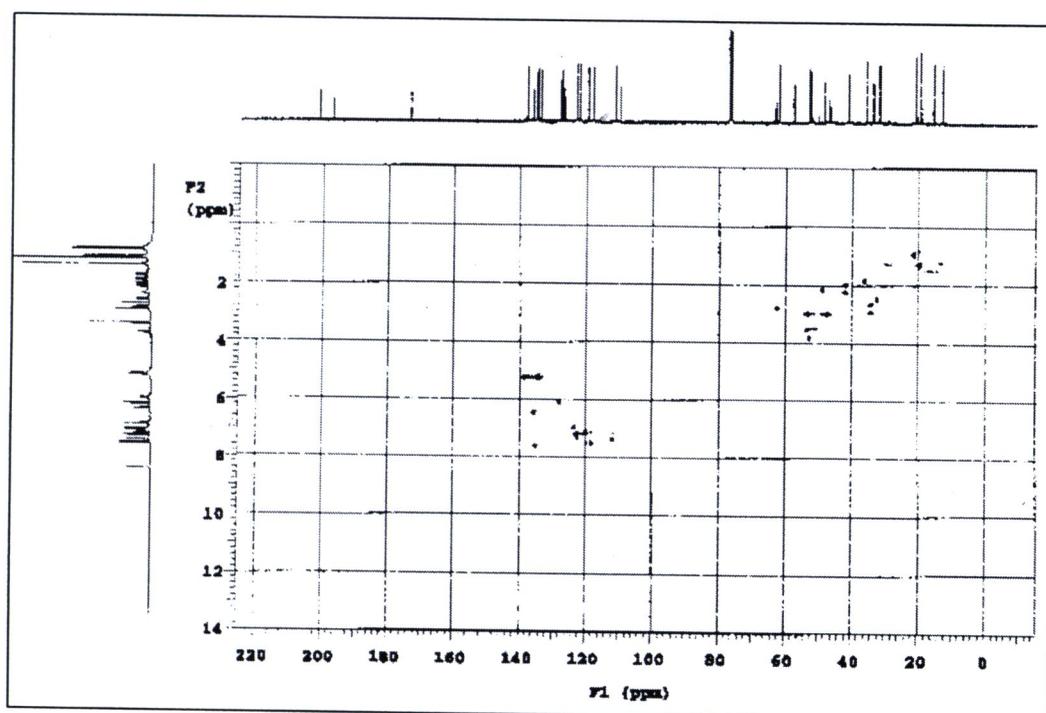


Figure 69 HSQC spectrum of prochaetoglobosin III (IX).

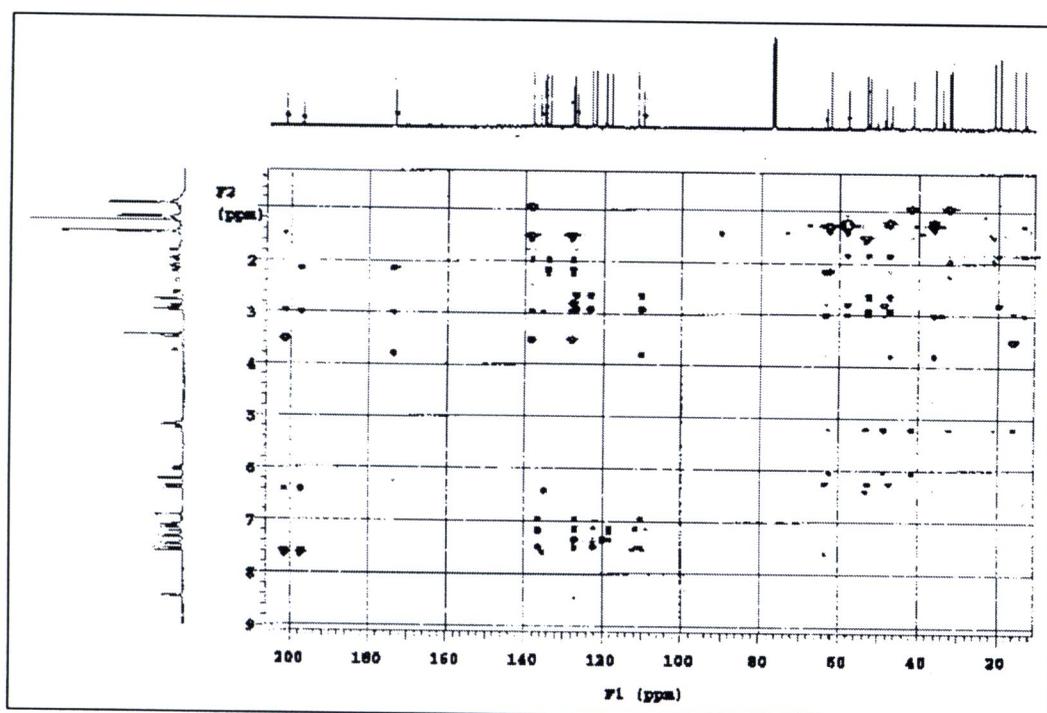


Figure 70 HMBC spectrum of prochaetoglobosin III (IX).

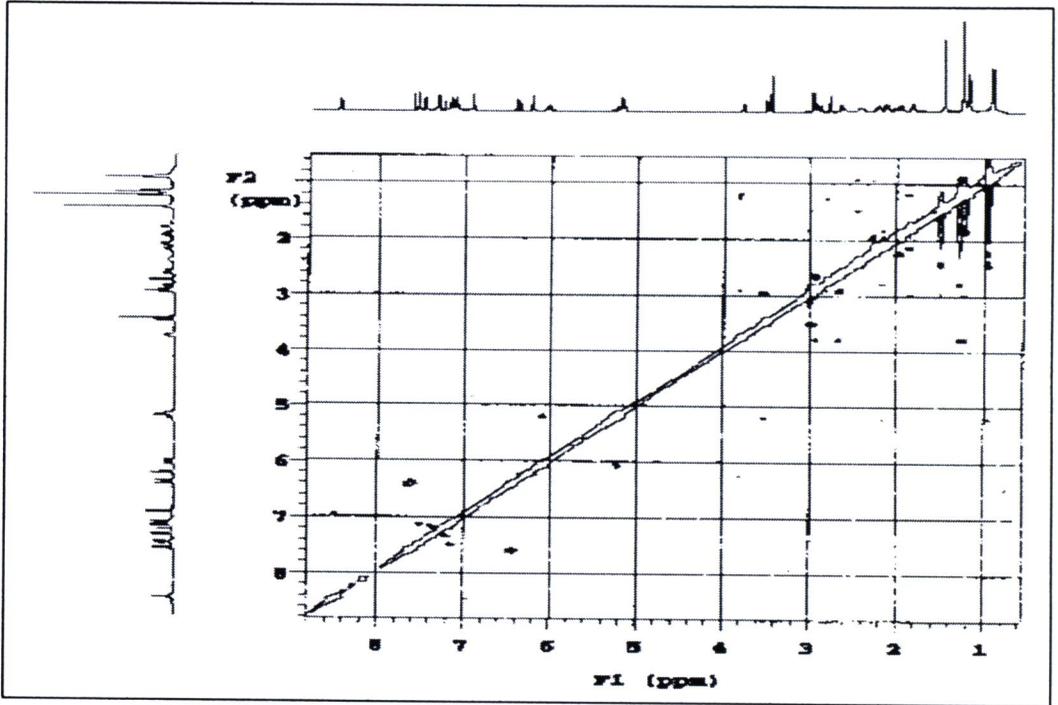


Figure 71 NOESY spectrum of prochaetoglobosin III (IX).

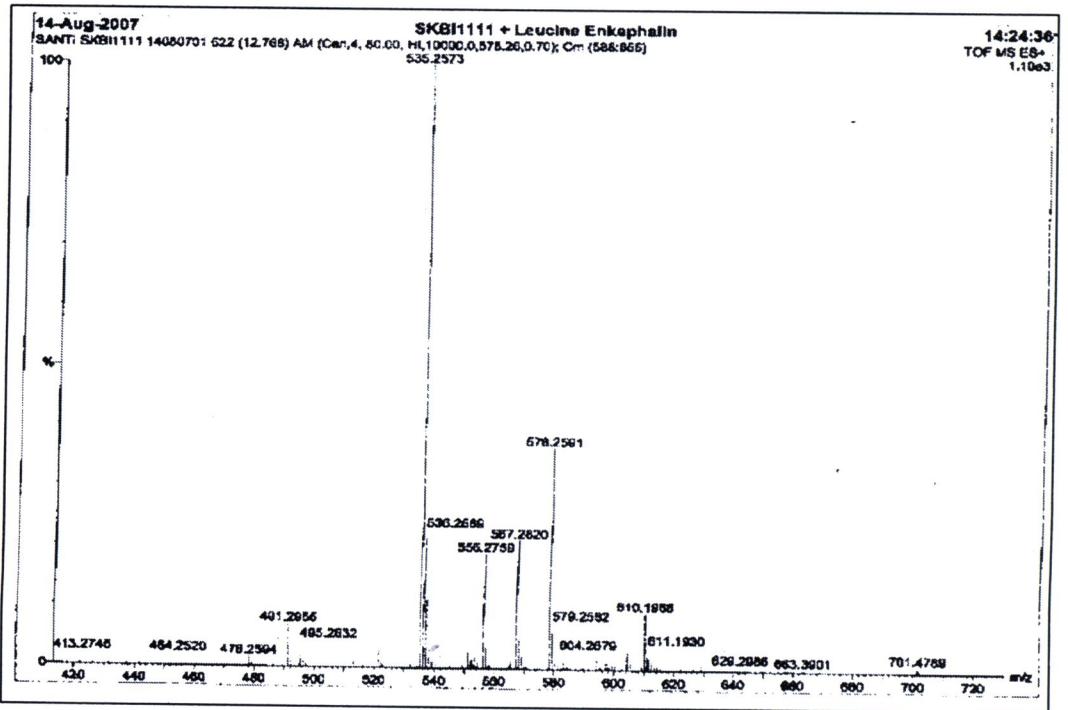


Figure 72 Mass spectrum of prochaetoglobosin III (X).

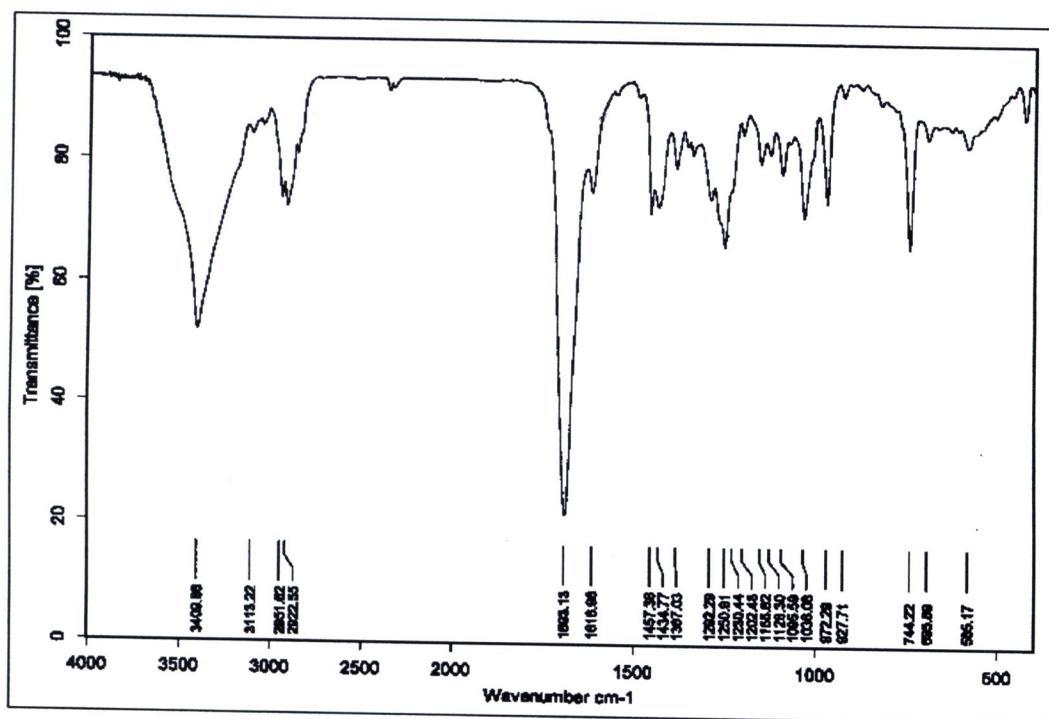


Figure 73 IR spectrum of prochaetoglobosin IIIed (X).

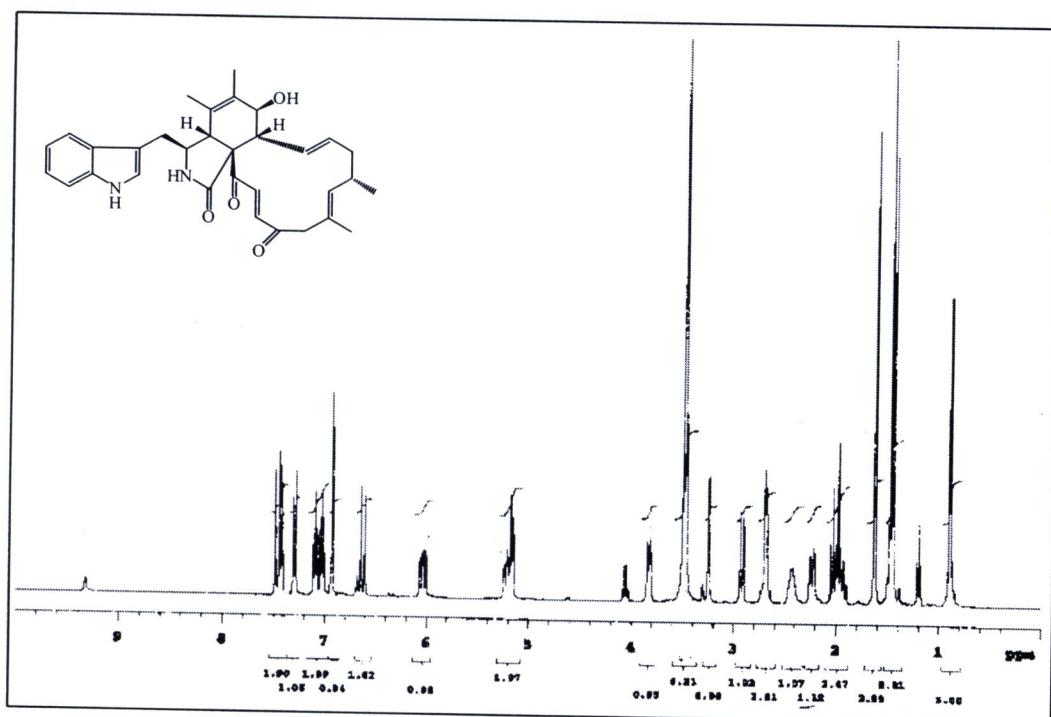


Figure 74 ^1H NMR spectrum of prochaetoglobosin IIIed (X).

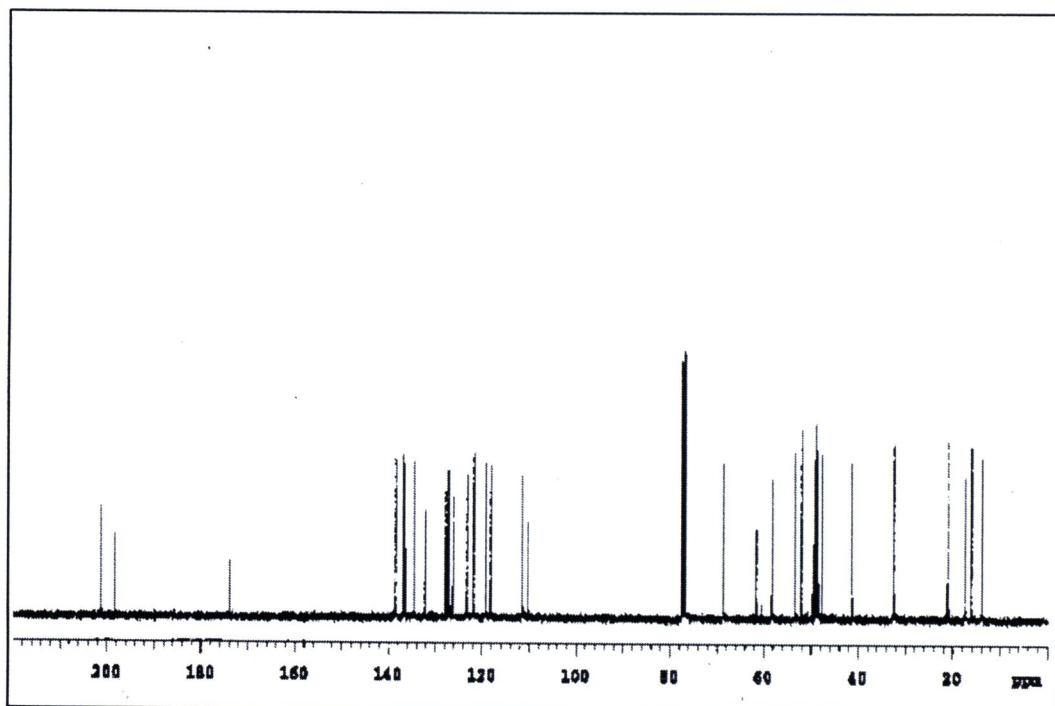


Figure 75 ^{13}C NMR spectrum of prochaetoglobosin IIIed (X).

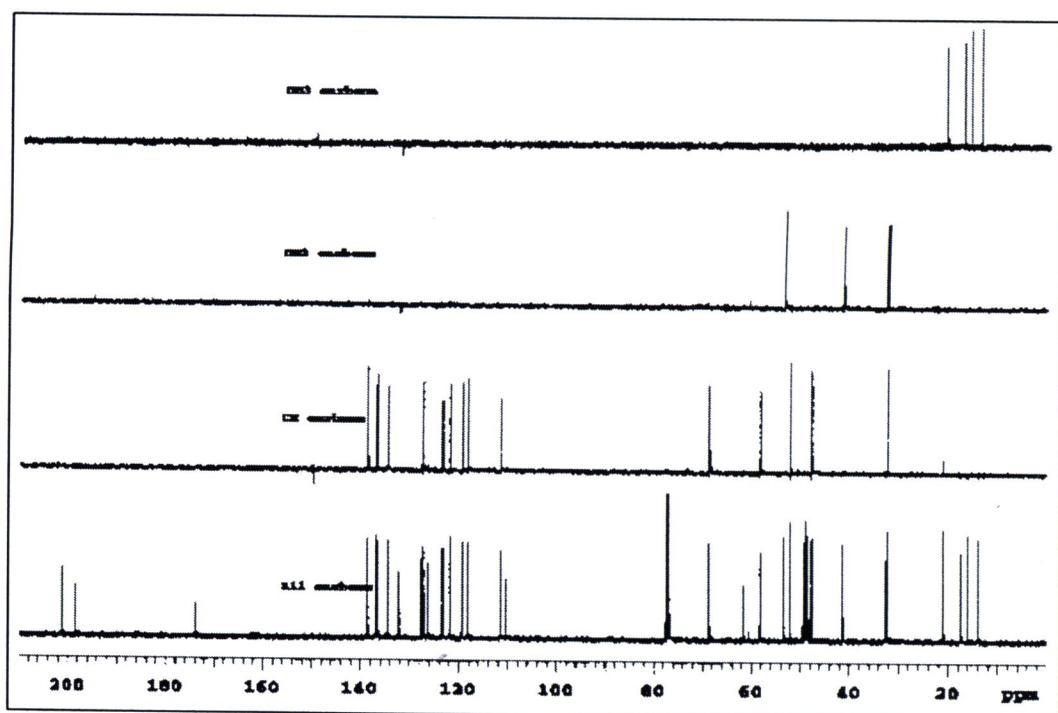


Figure 76 DEPT spectrum of prochaetoglobosin IIIed (X).

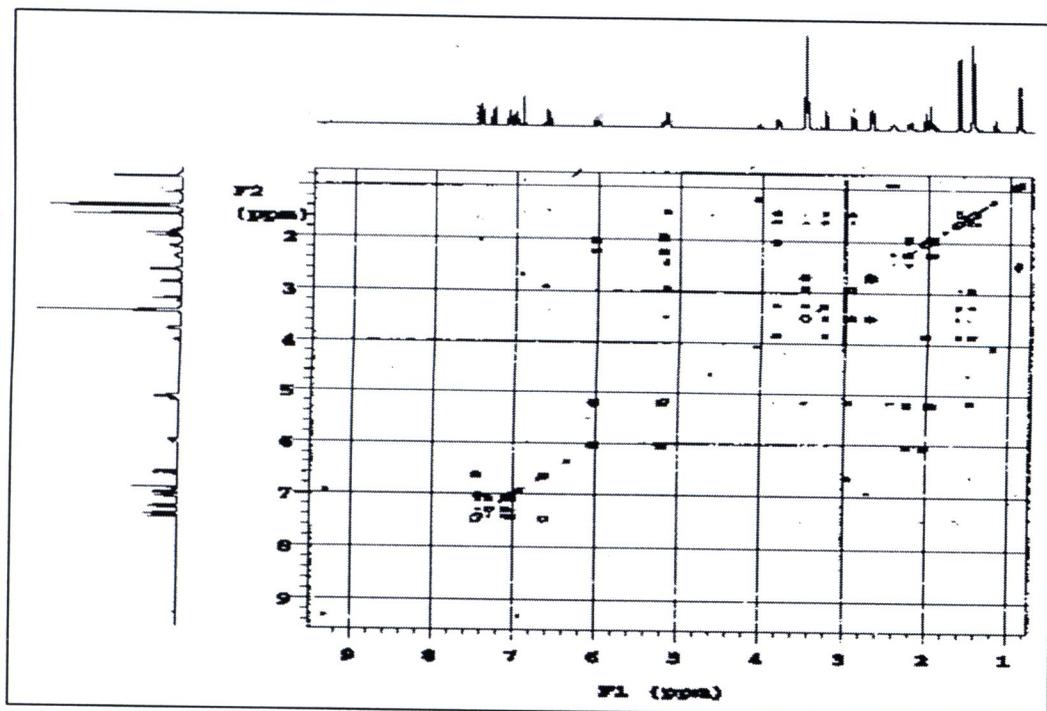


Figure 77 COSY spectrum of prochaetoglobosin IIIed (X).

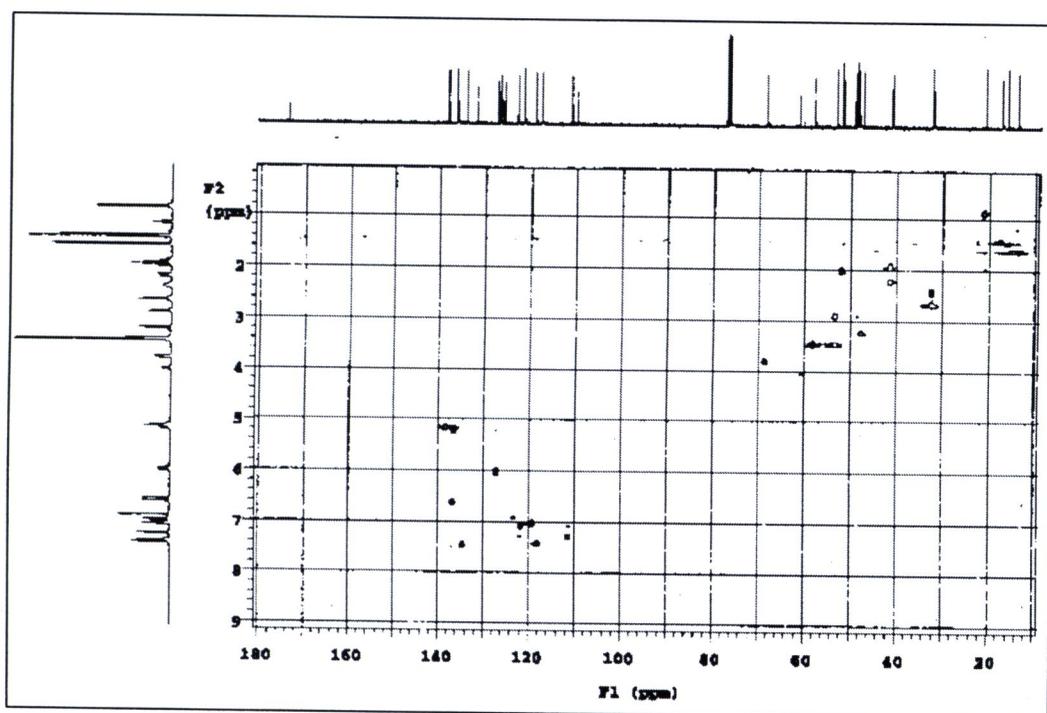


Figure 78 HSQC spectrum of prochaetoglobosin IIIed (X).

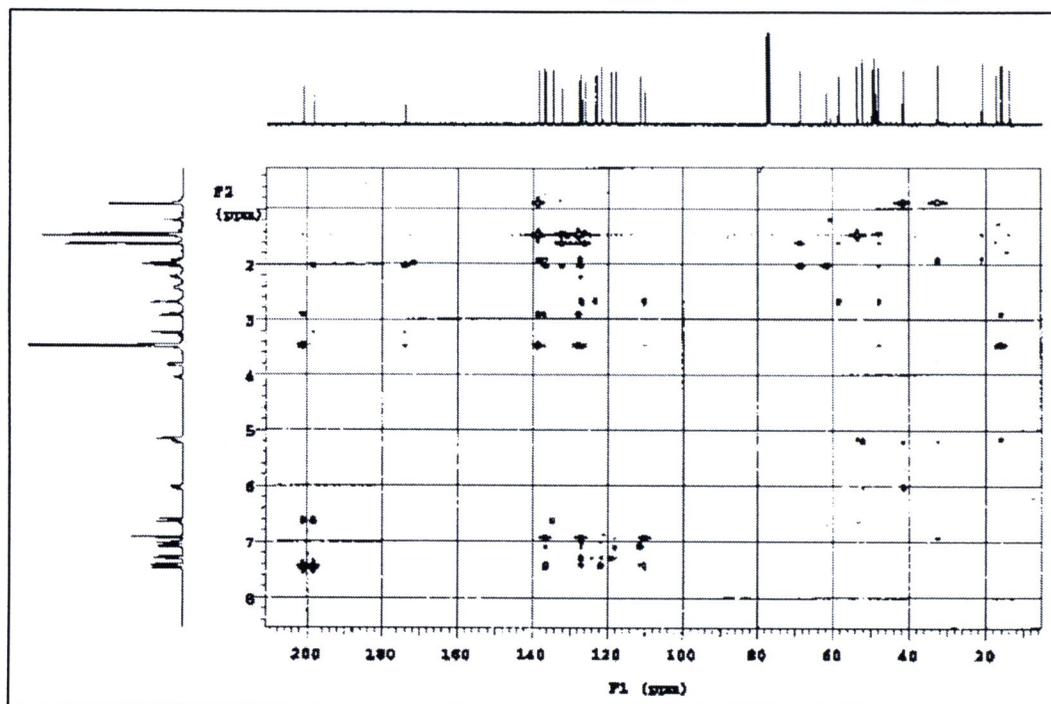


Figure 79 HMBC spectrum of prochaetoglobosin IIIed (X).

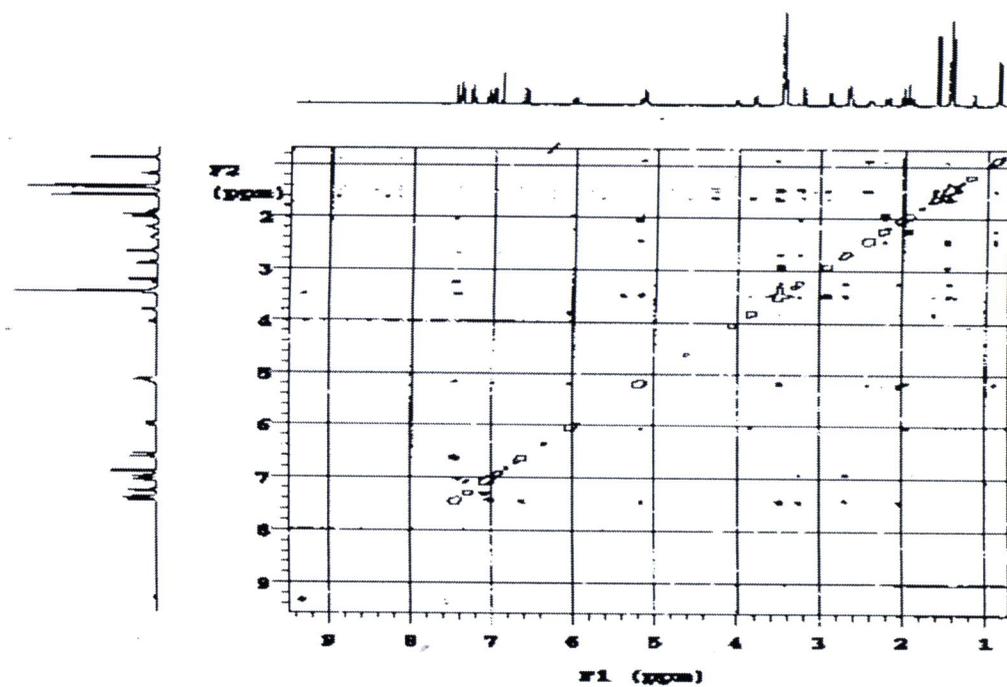


Figure 80 NOESY spectrum of prochaetoglobosin IIIed (X).

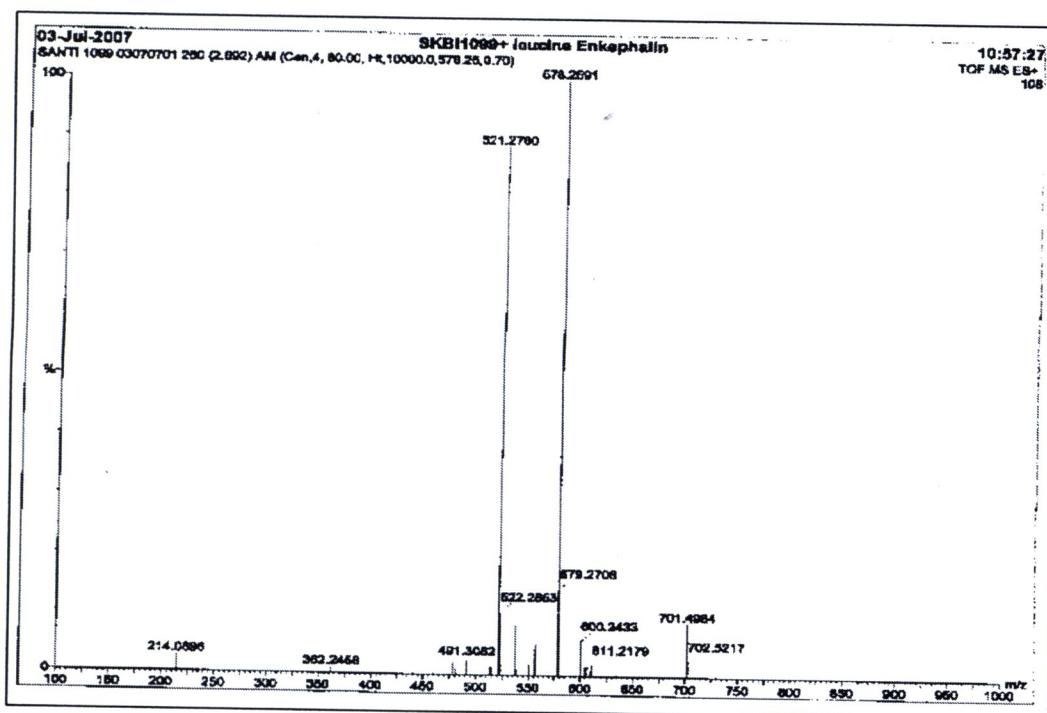


Figure 81 Mass spectrum of chaetoglobosin V (XI).

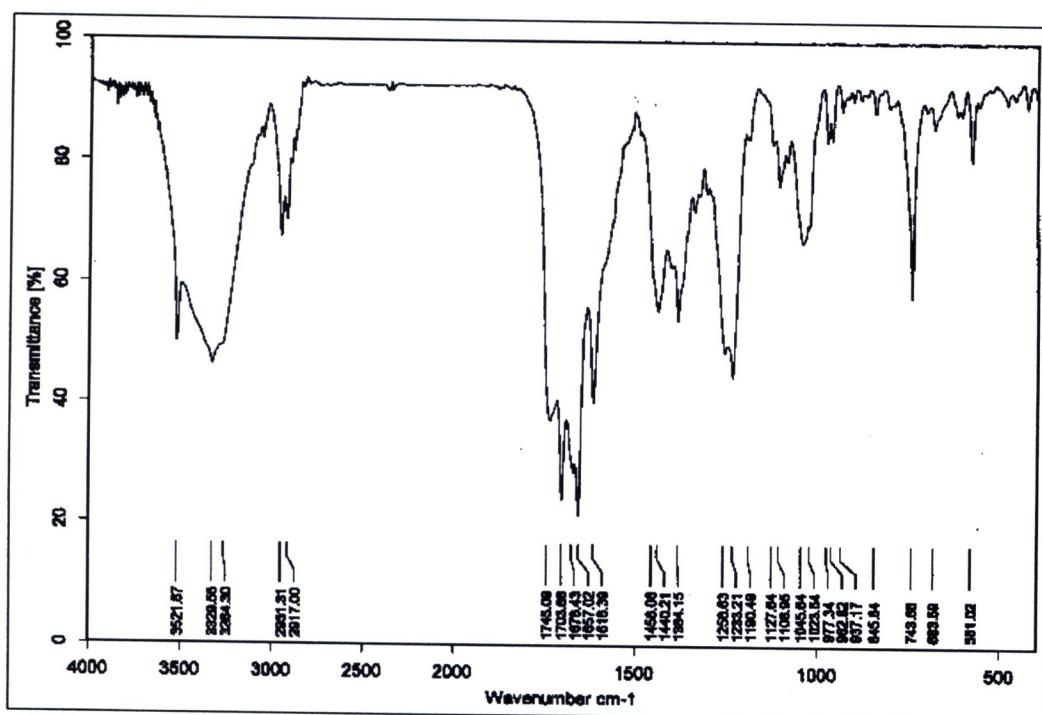


Figure 82 IR spectrum of chaetoglobosin V (XI).

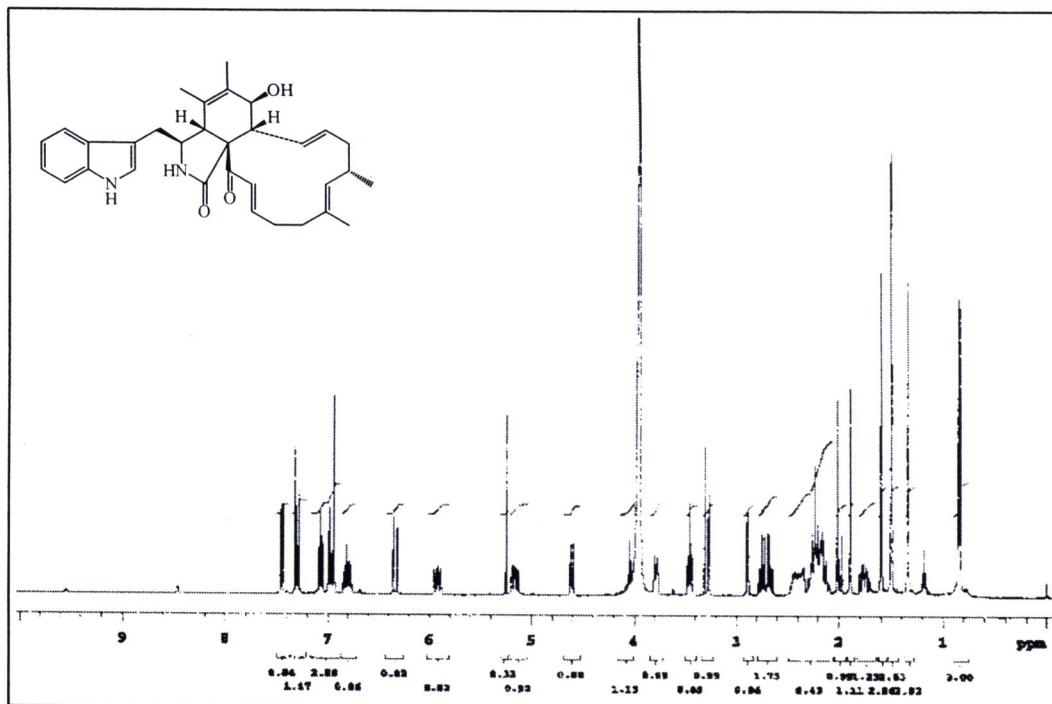


Figure 83 ^1H NMR spectrum of chaetoglobosin V (XI).

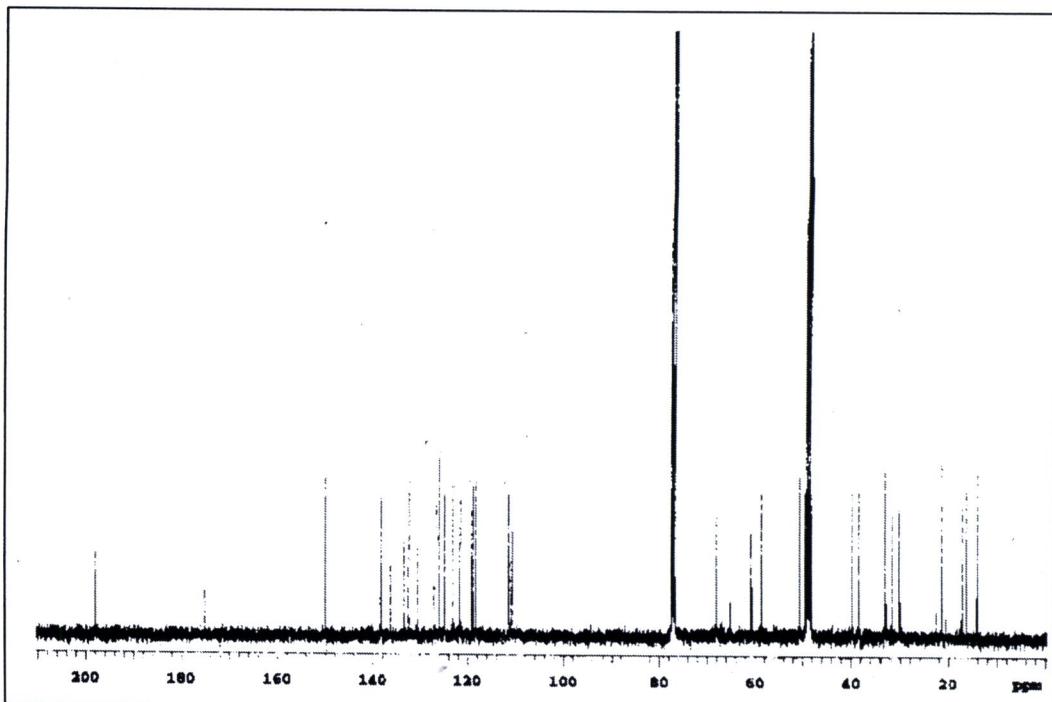


Figure 84 ^{13}C NMR spectrum of chaetoglobosin V (XI).

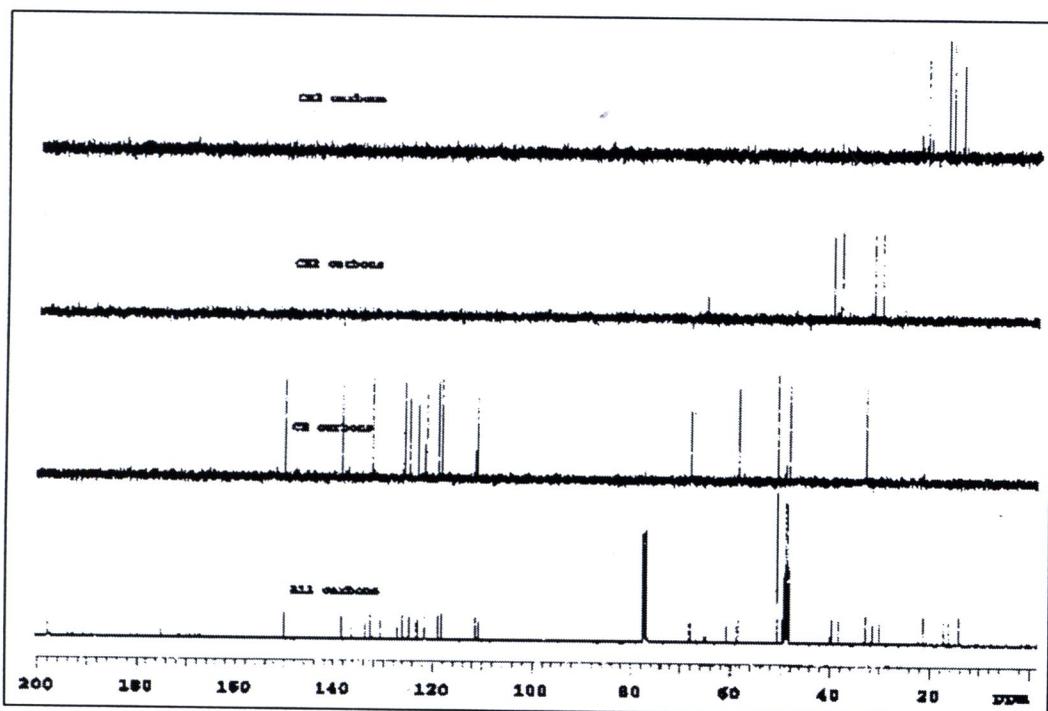


Figure 85 DEPT spectrum of chaetoglobosin V (XI).

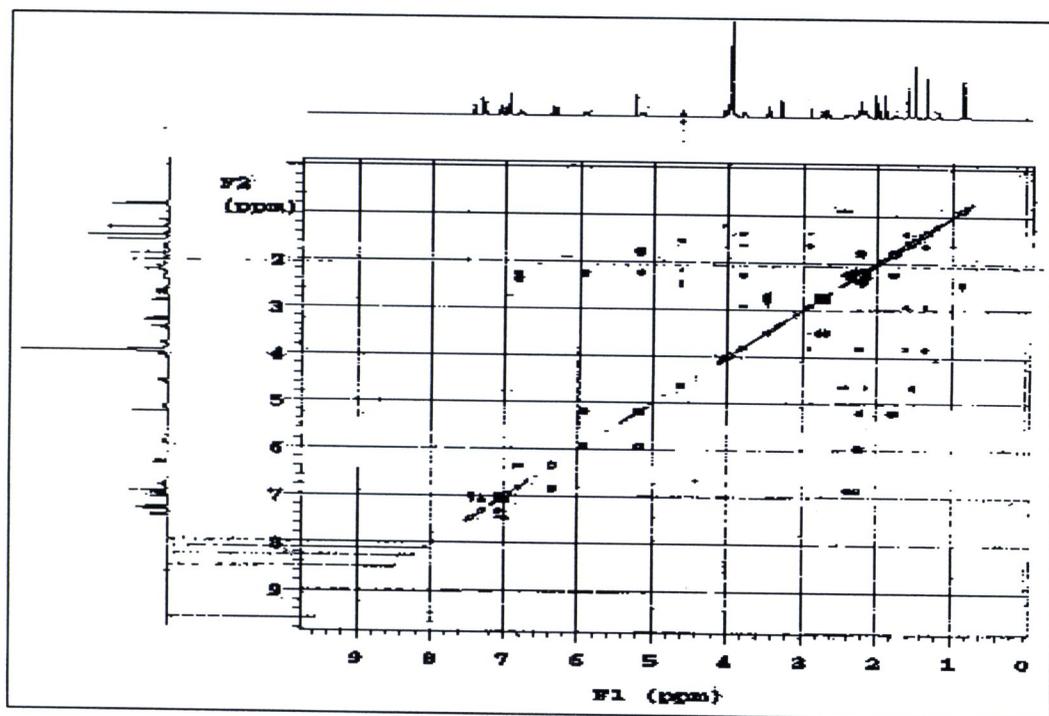


Figure 86 COSY spectrum of chaetoglobosin V (XI).

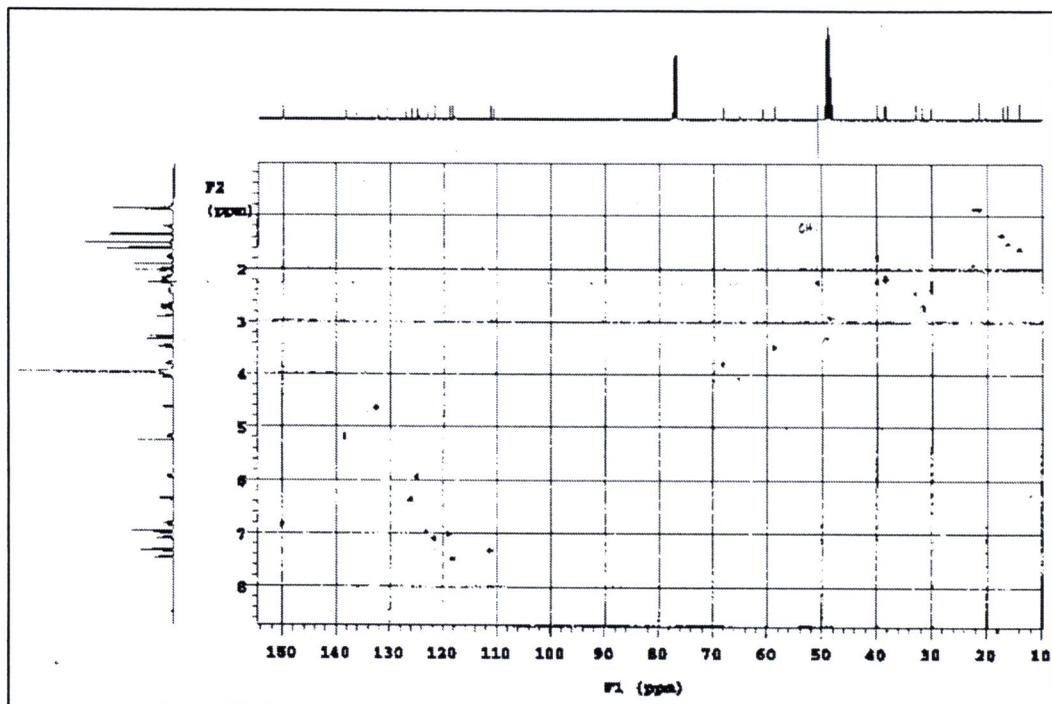


Figure 87 HSQC spectrum of chaetoglobosin V (XI).

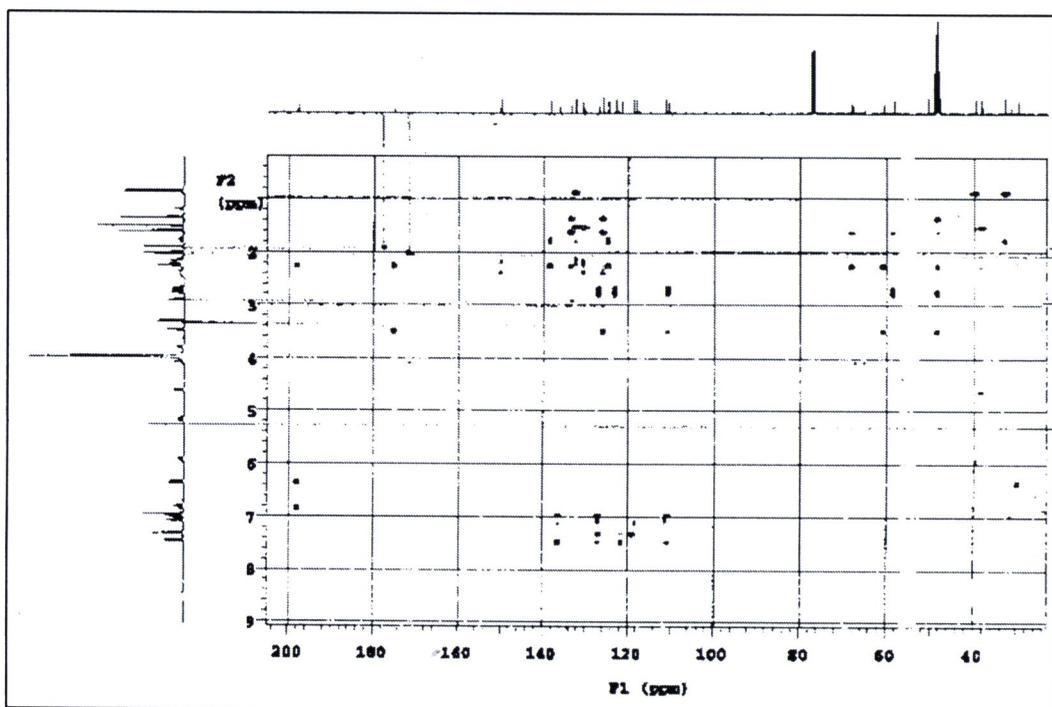


Figure 88 HMBC spectrum of chaetoglobosin V (XI).

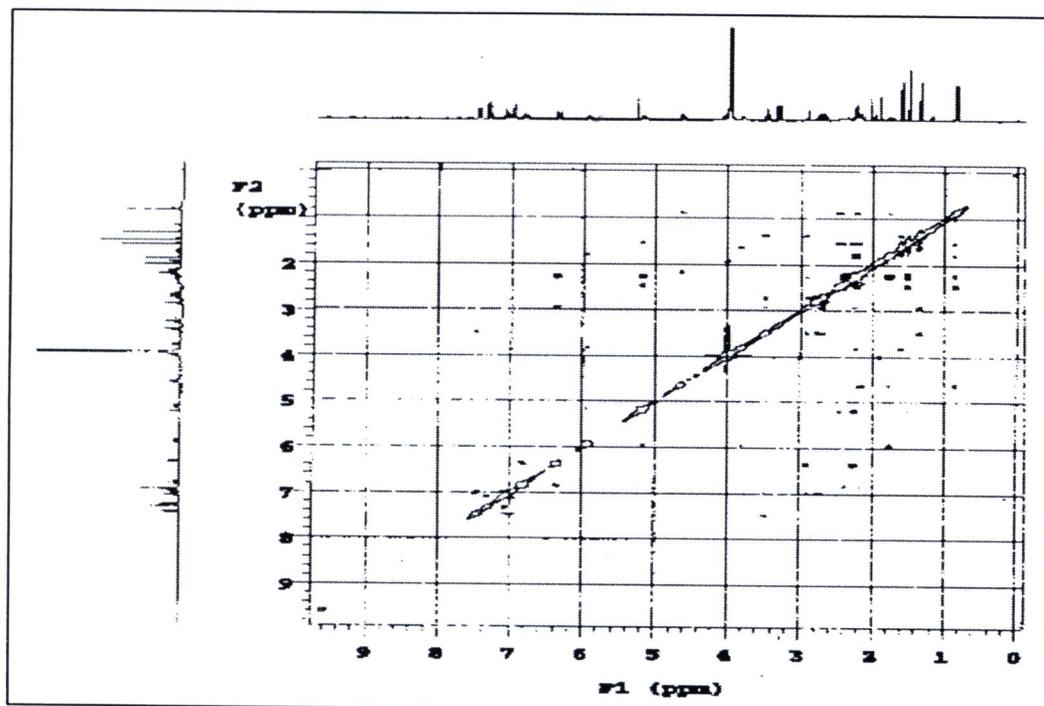


Figure 89 NOESY spectrum of chaetoglobosin V (XI).

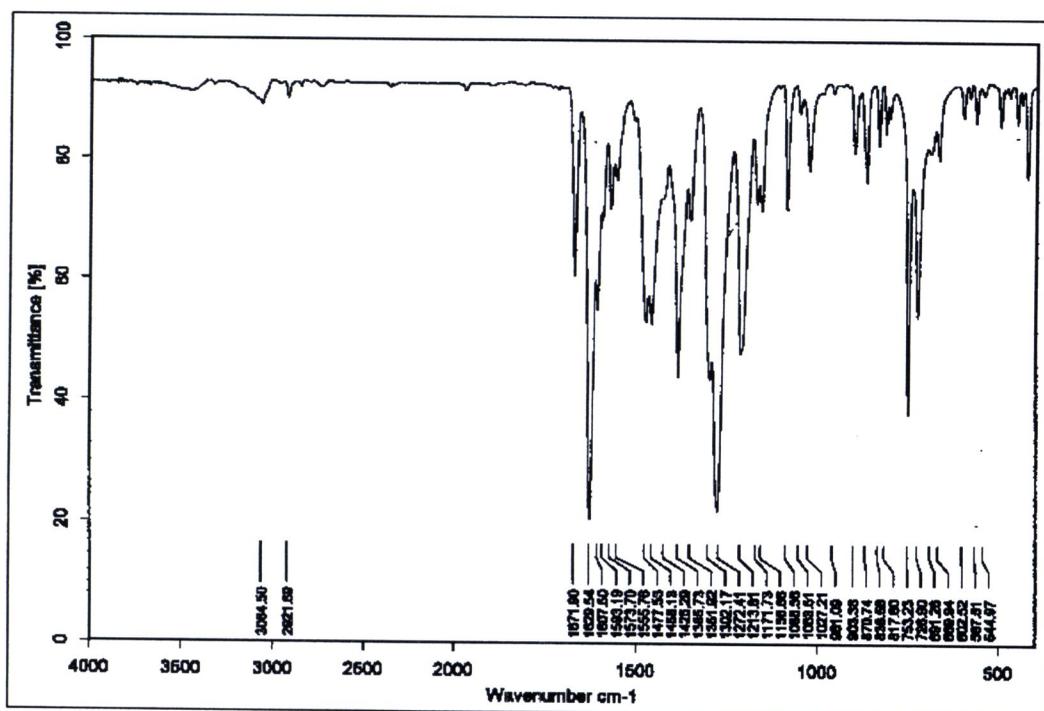


Figure 90 IR spectrum of chrysophanol (XII).

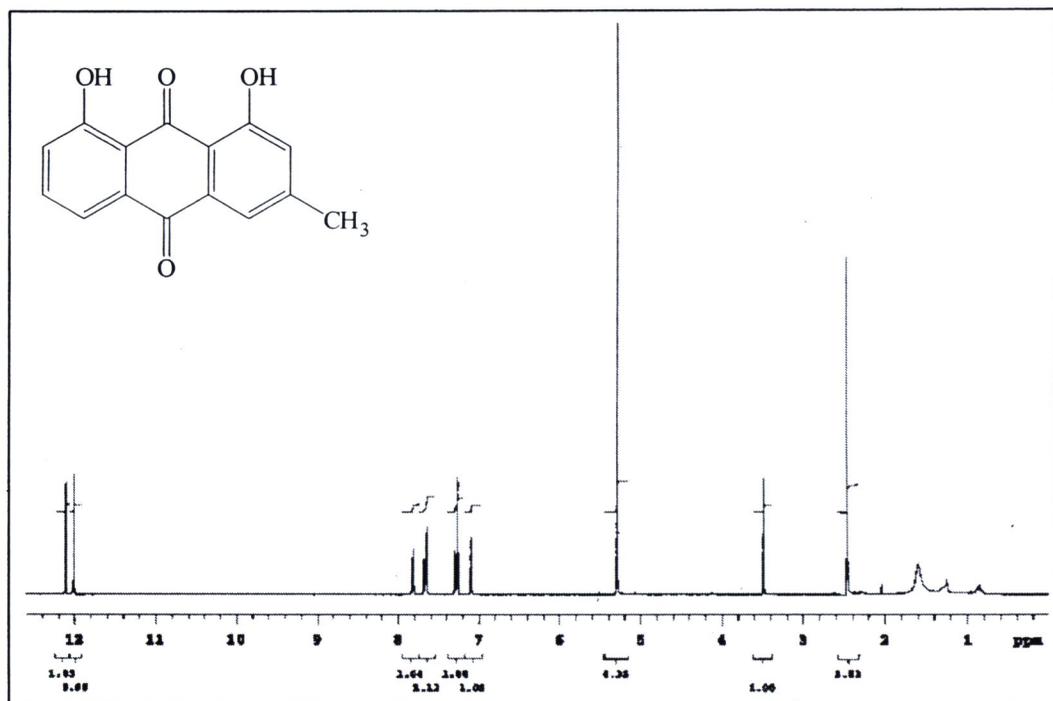


Figure 91 ¹H NMR spectrum of chrysophanol (XII).

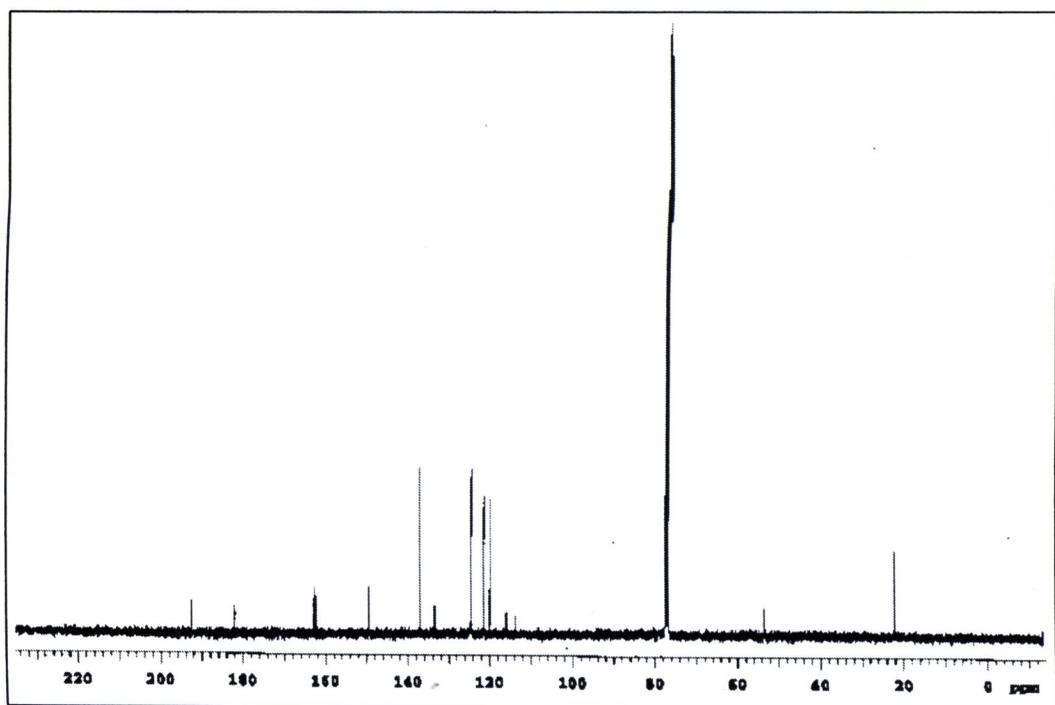


Figure 92 ¹³C NMR spectrum of chrysophanol (XII).

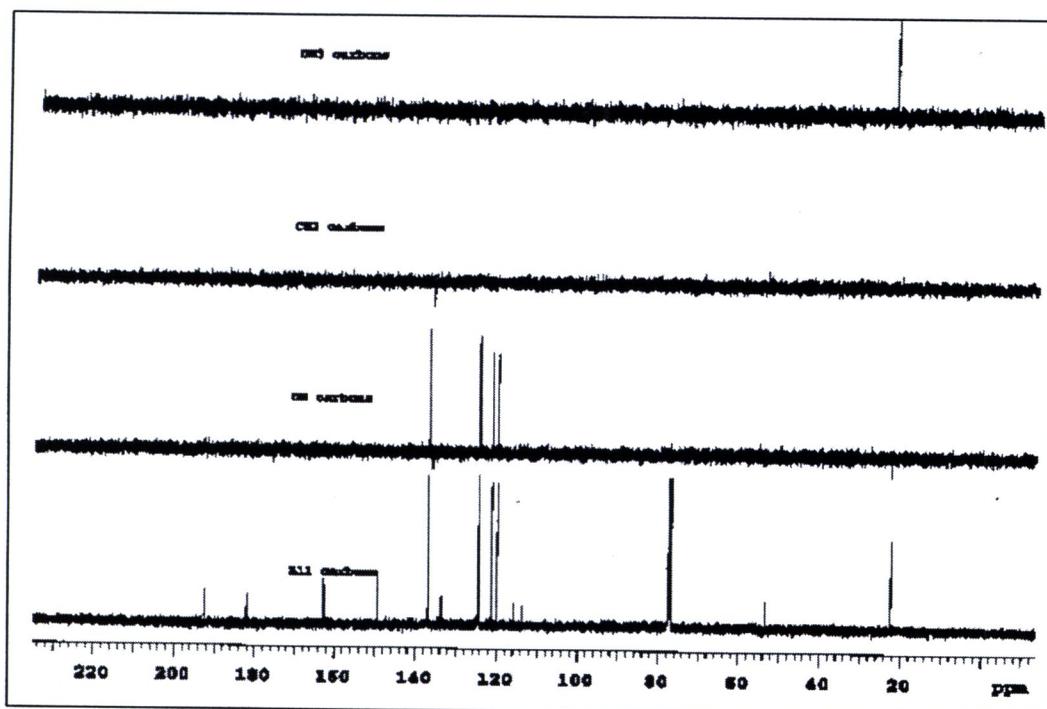


Figure 93 DEPT spectrum of chrysophanol (XII).

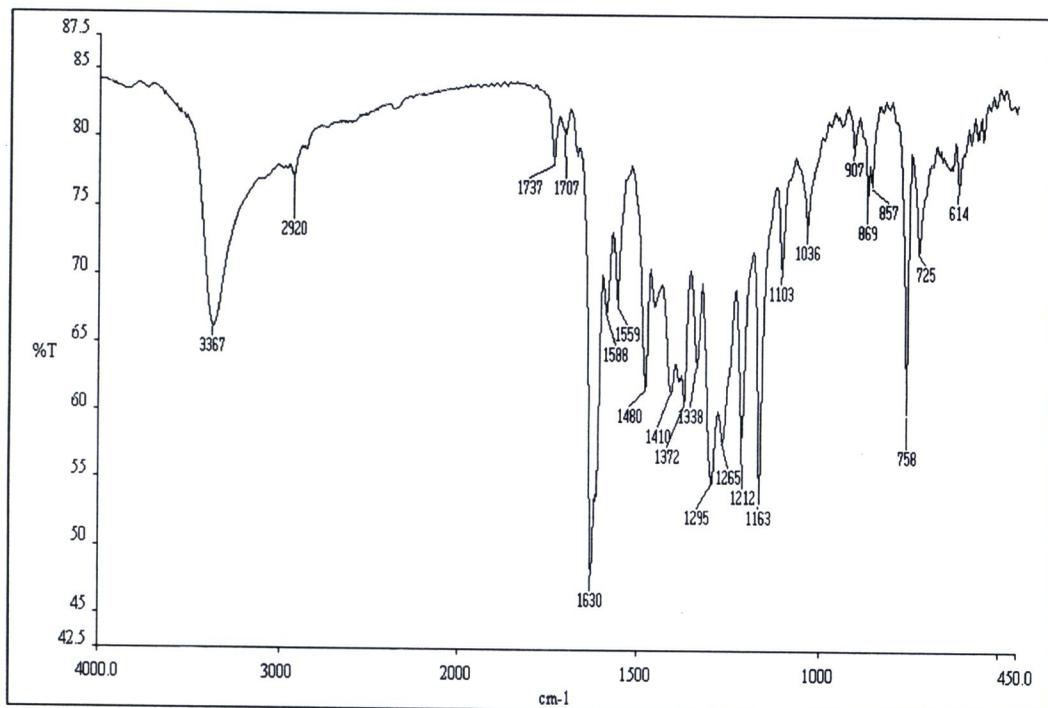


Figure 94 IR spectrum of emodin (XIII).

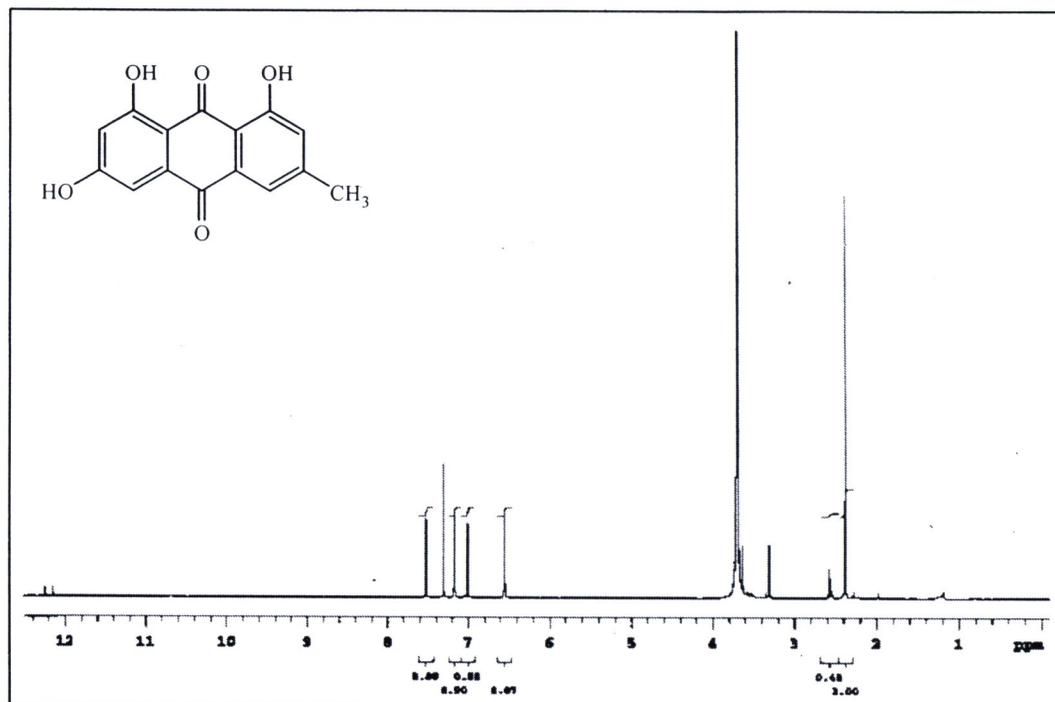


Figure 95 ¹H NMR spectrum of emodin (XIII).

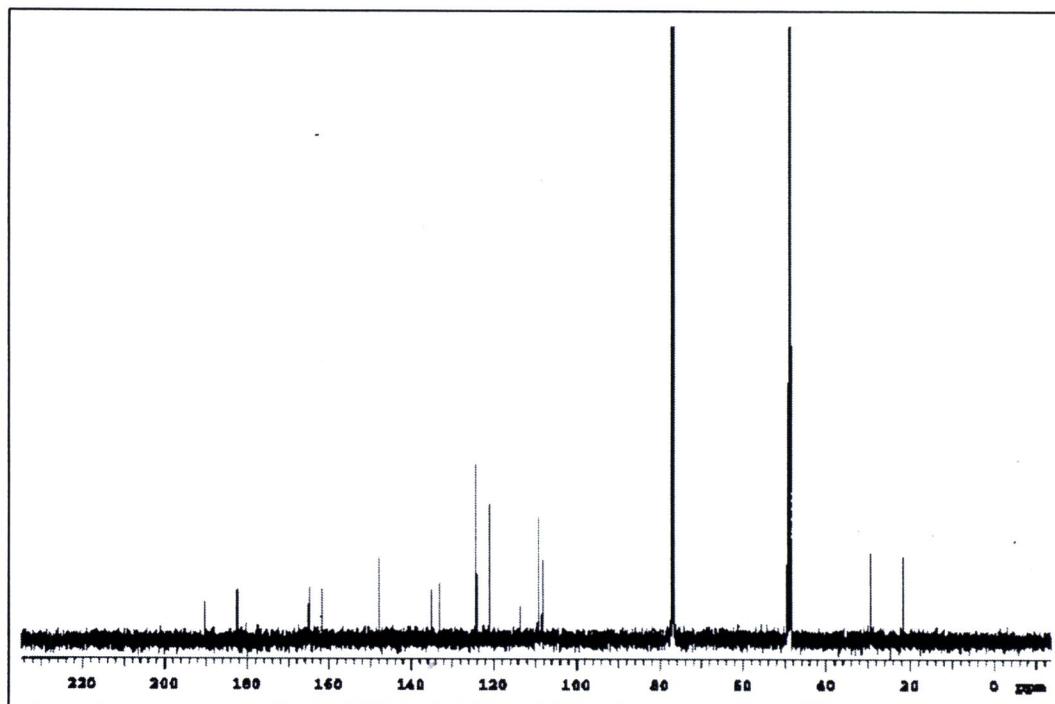


Figure 96 ¹³C NMR spectrum of emodin (XIII).

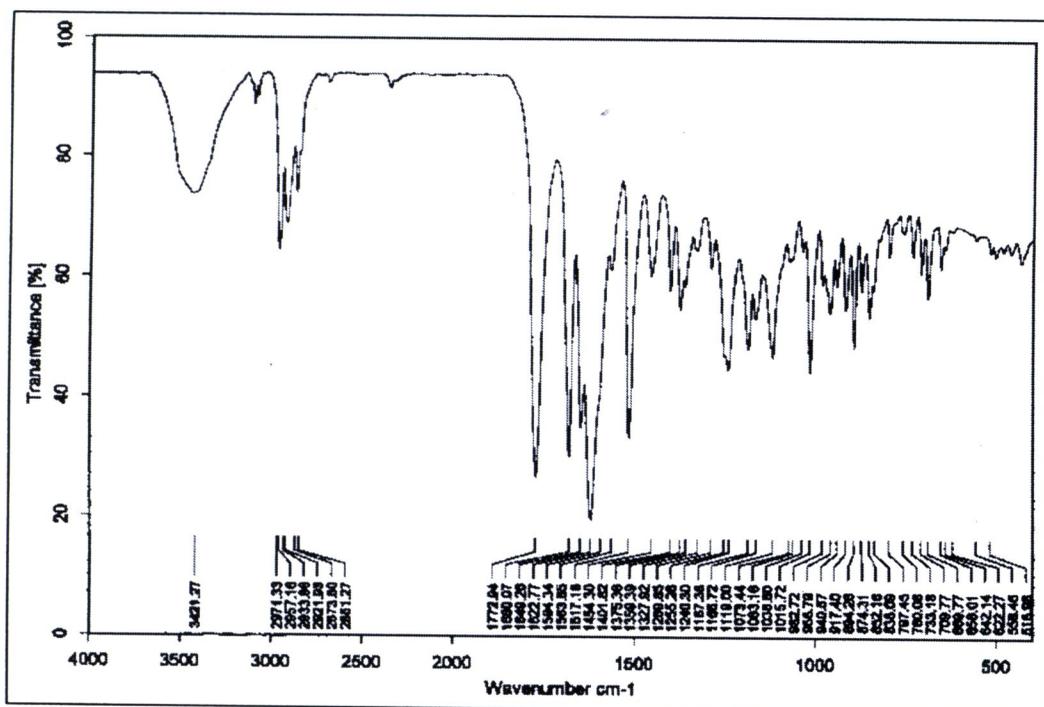


Figure 97 IR spectrum of chaetoviridin A (XIV).

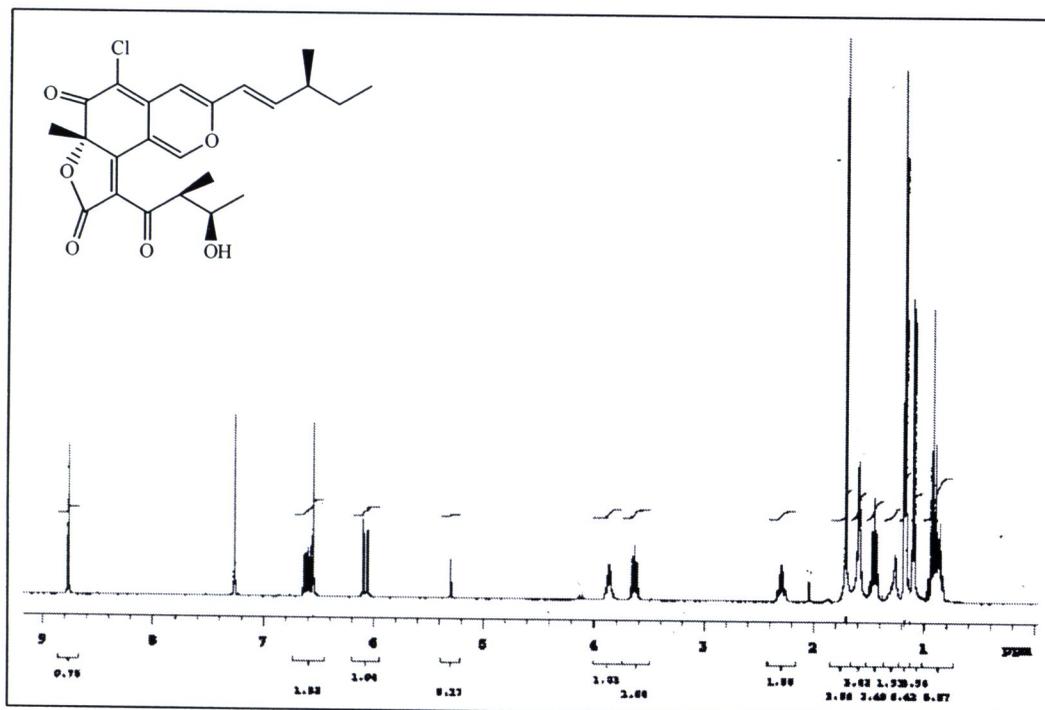


Figure 98 ¹H NMR spectrum of chaetoviridin A (XIV).

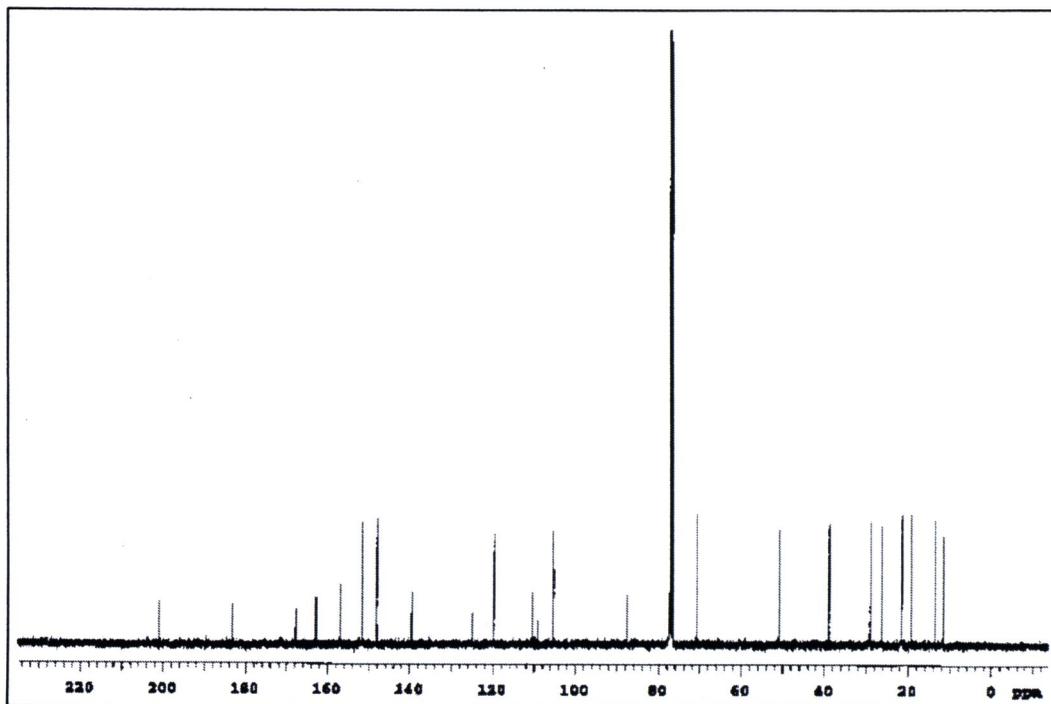


Figure 99 ^{13}C NMR spectrum of chaetoviridin A (XIV).

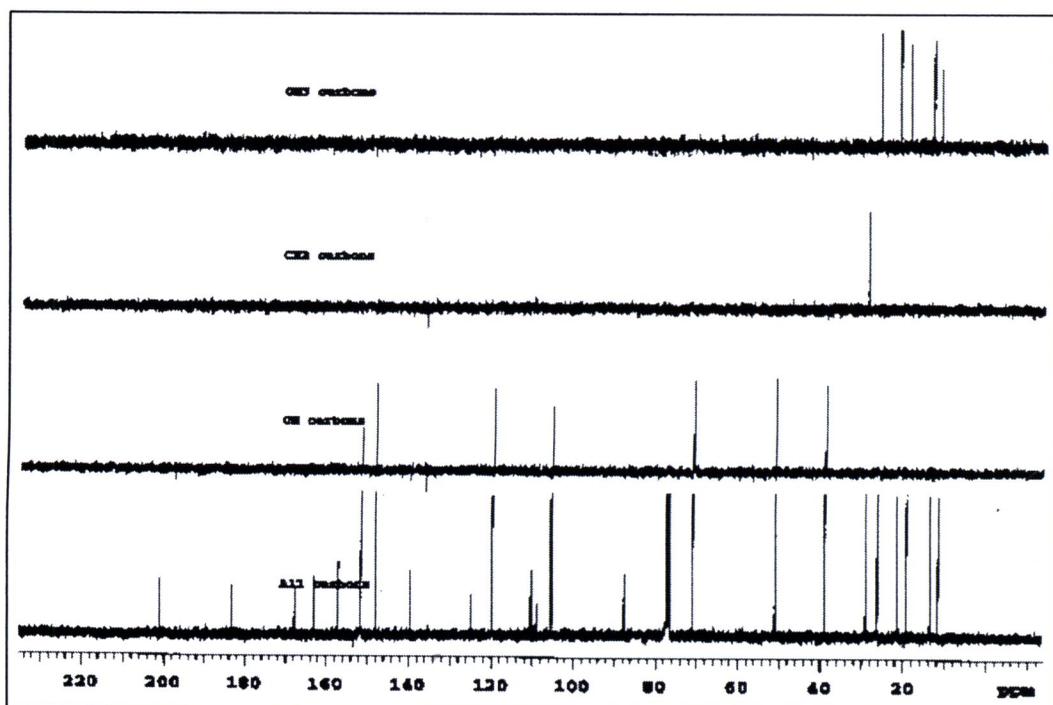


Figure 100 DEPT spectrum of chaetoviridin A (XIV).

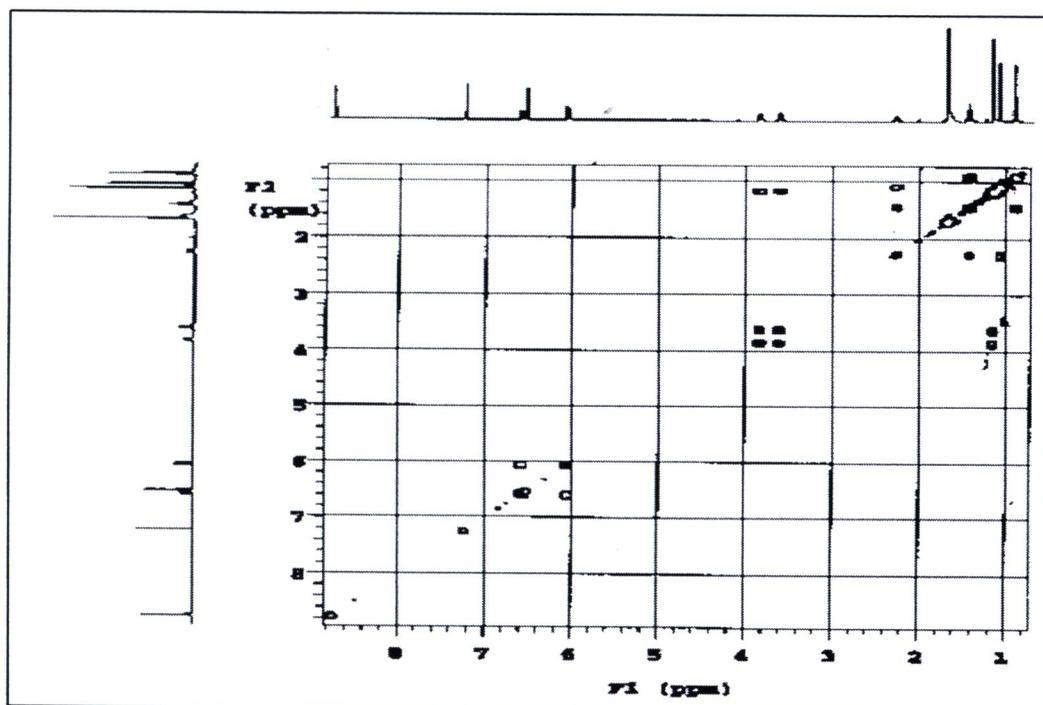


Figure 101 COSY spectrum of chaetoviridin A (XIV).

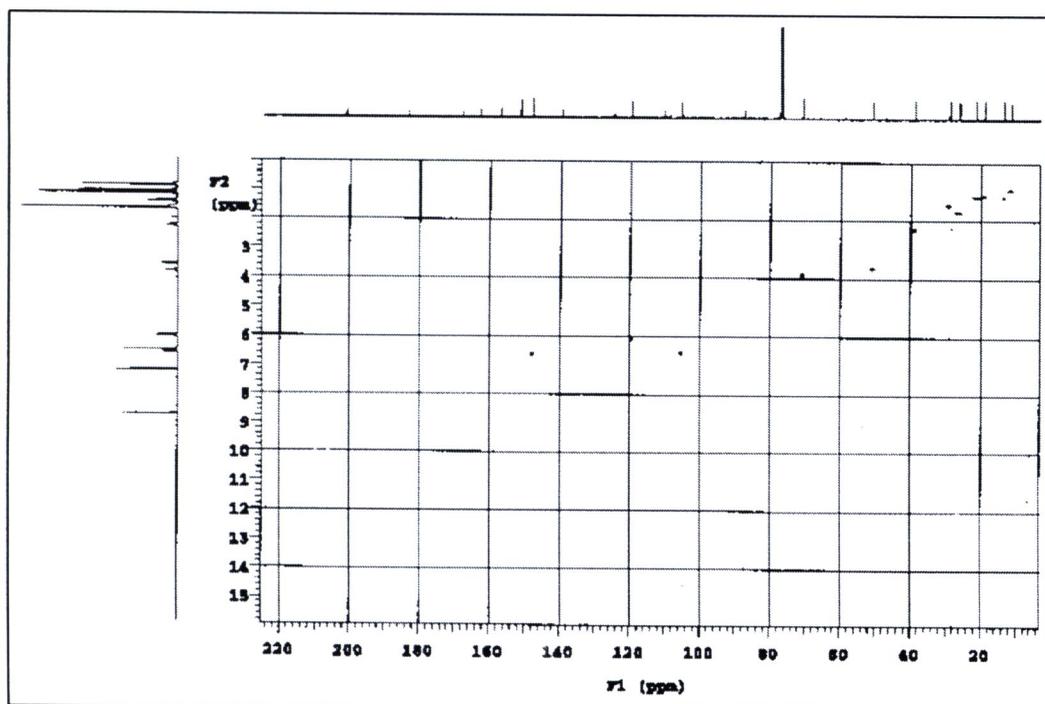


Figure 102 HSQC spectrum of chaetoviridin A (XIV).

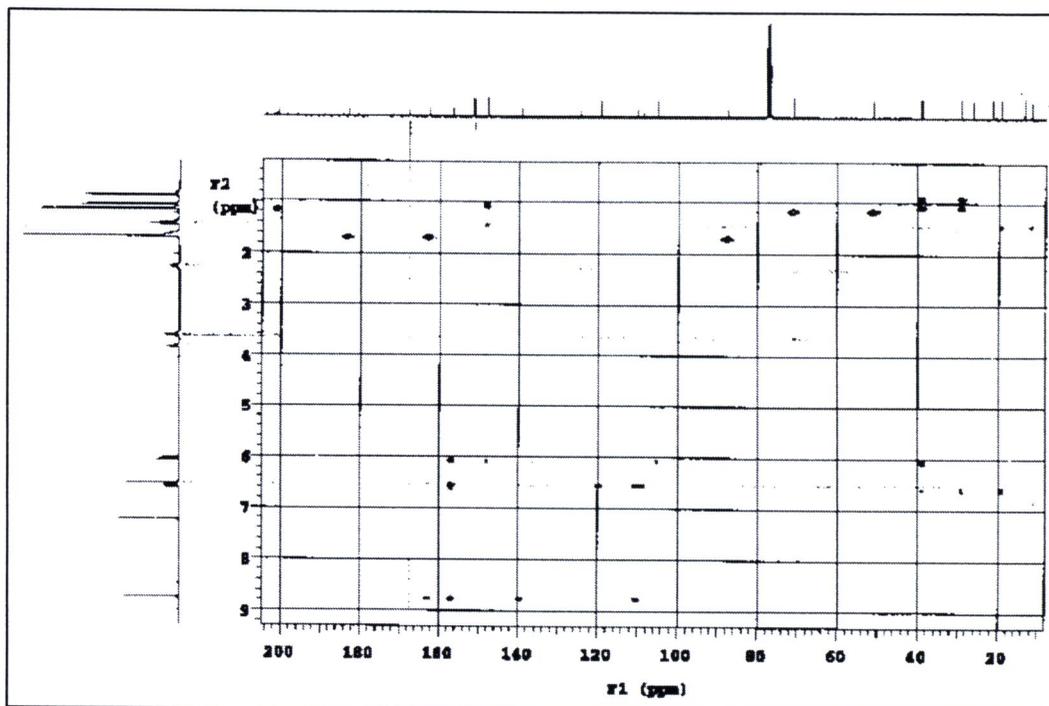


Figure 103 HMBC spectrum of chaetoviridin A (XIV).

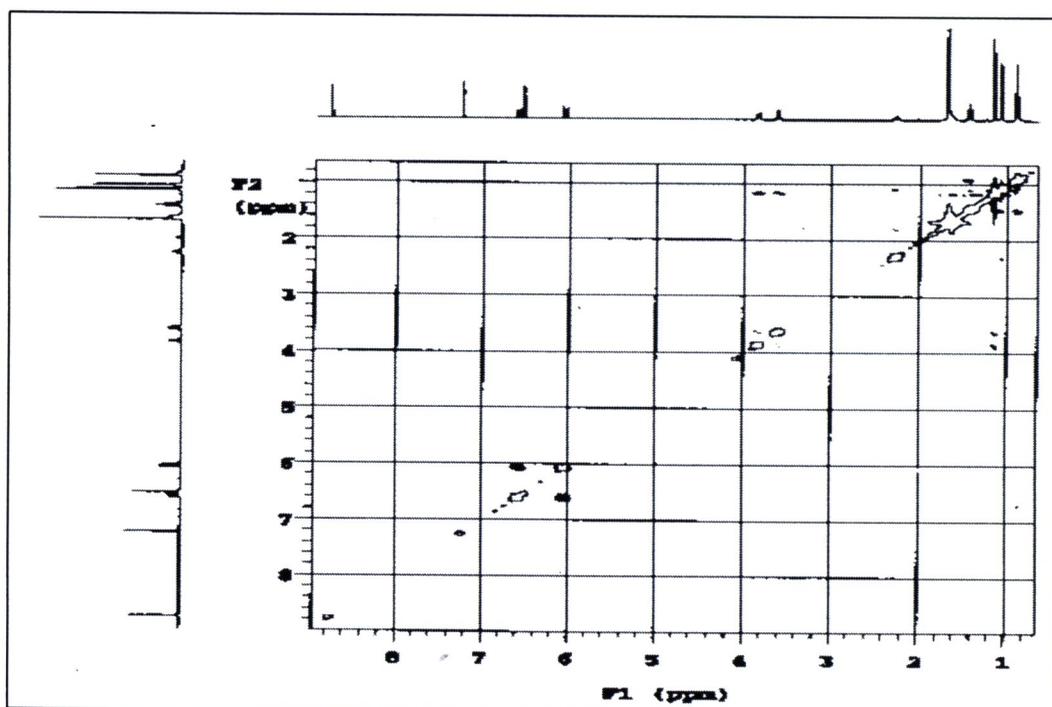


Figure 104 NOESY spectrum of chaetoviridin A (XIV).

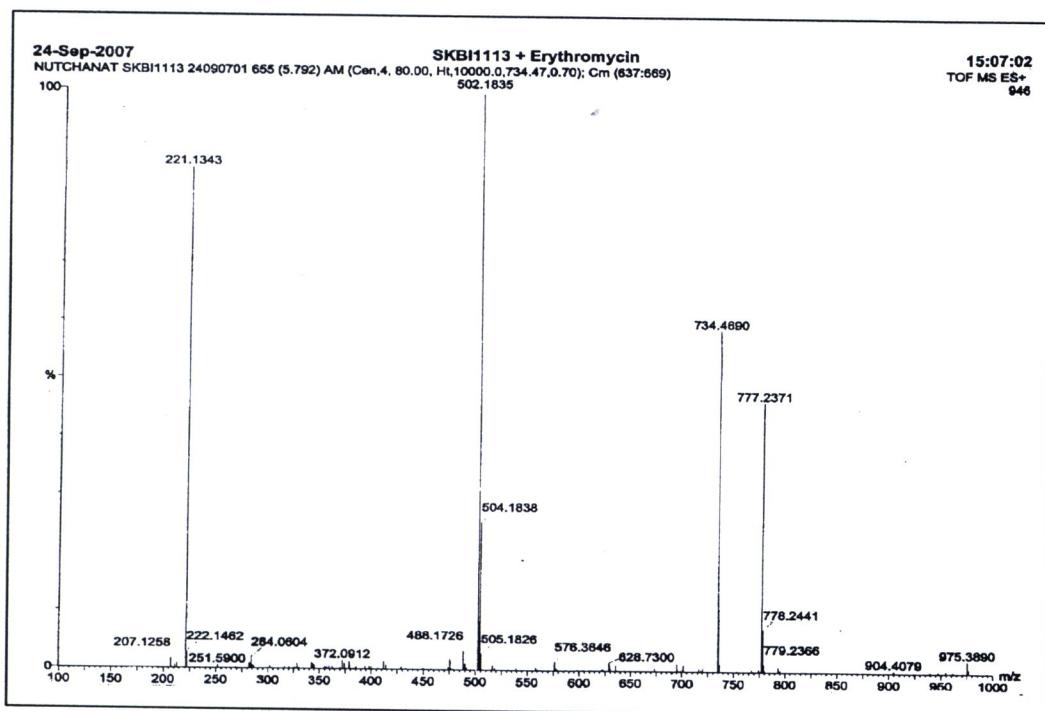


Figure 105 Mass spectrum of cochliodone D (XV).

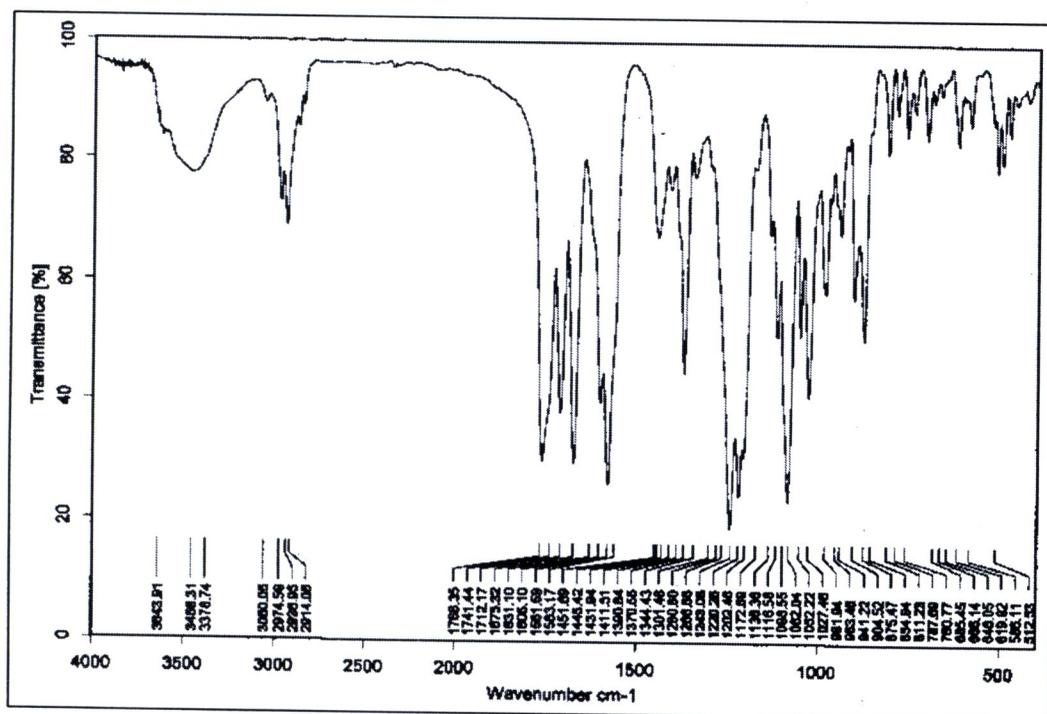


Figure 106 IR spectrum of cochliodone D (XV).

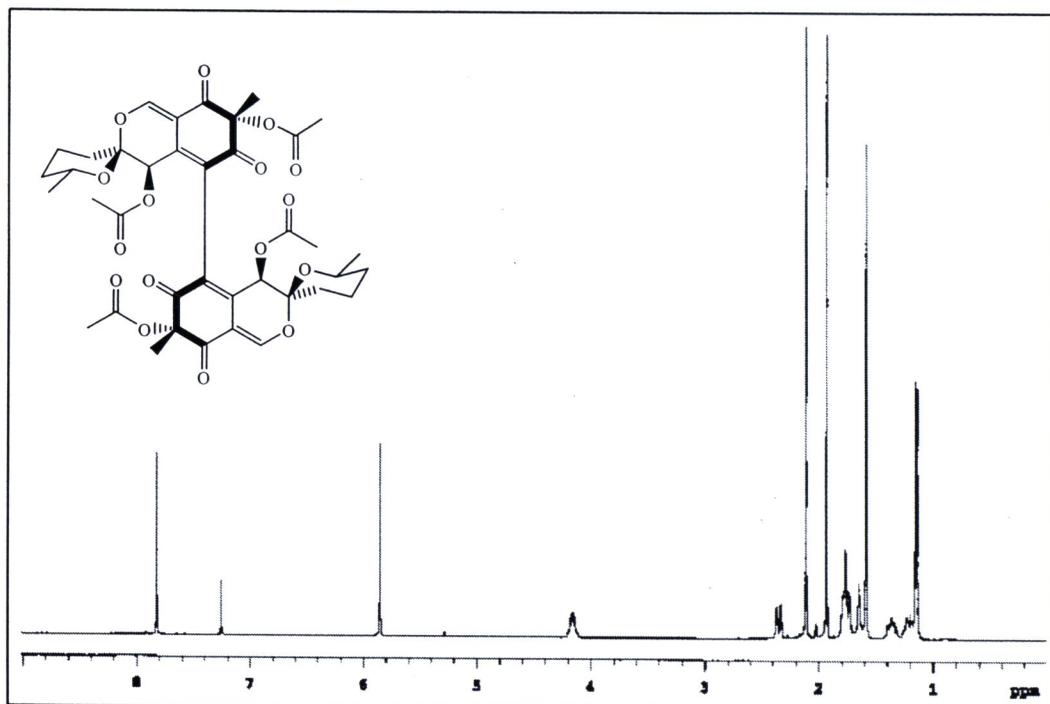


Figure 107 ¹H NMR spectrum of cochliodone D (XV).

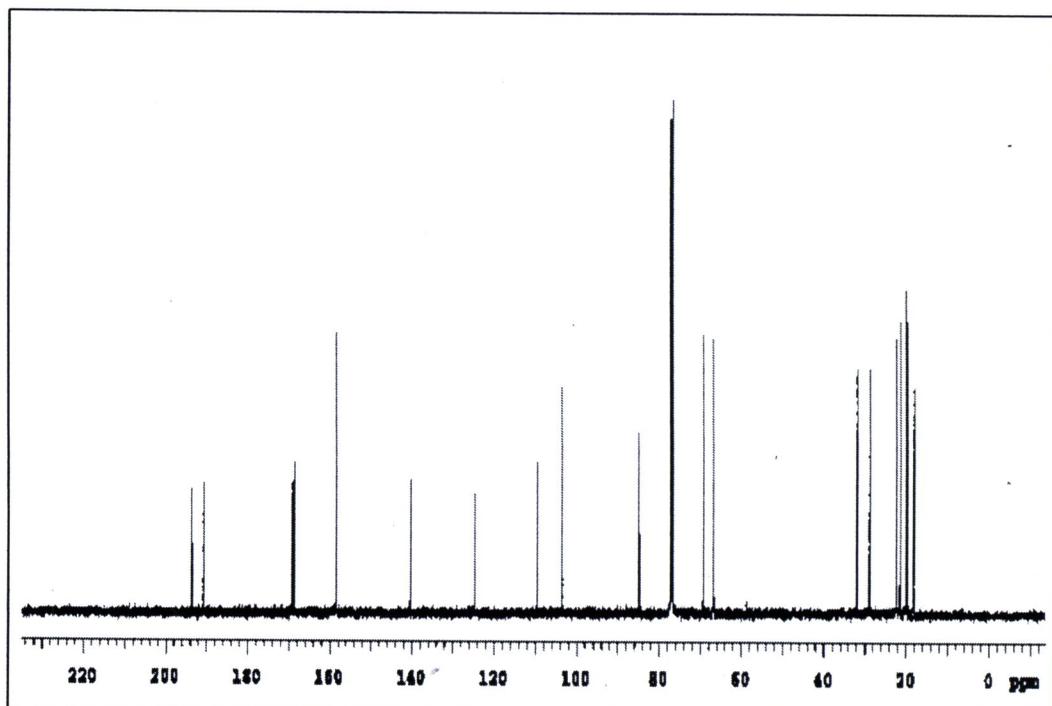


Figure 108 ¹³C NMR spectrum of cochliodone D (XV).

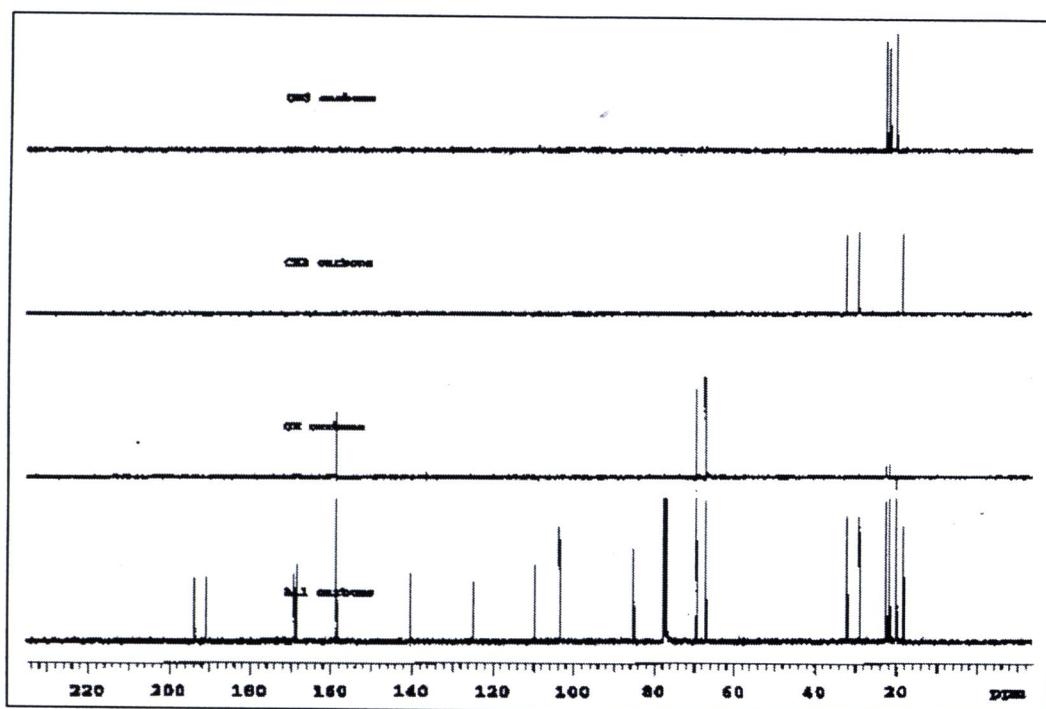


Figure 109 DEPT spectrum of cochliodone D (XV).

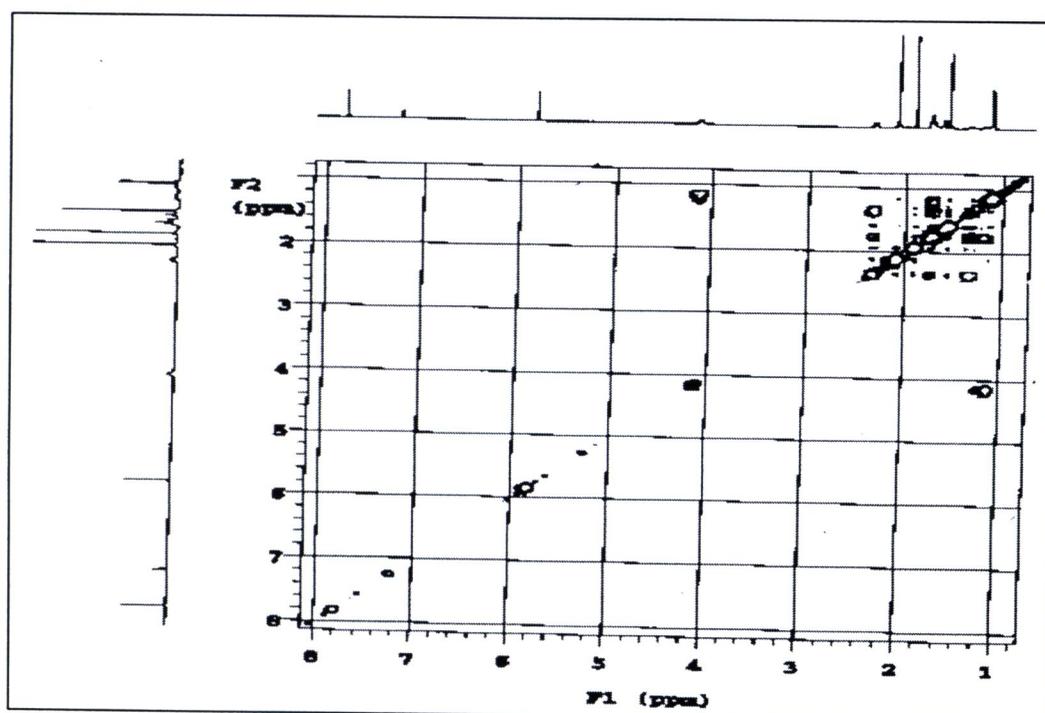


Figure 110 COSY spectrum of cochliodone D (XV).

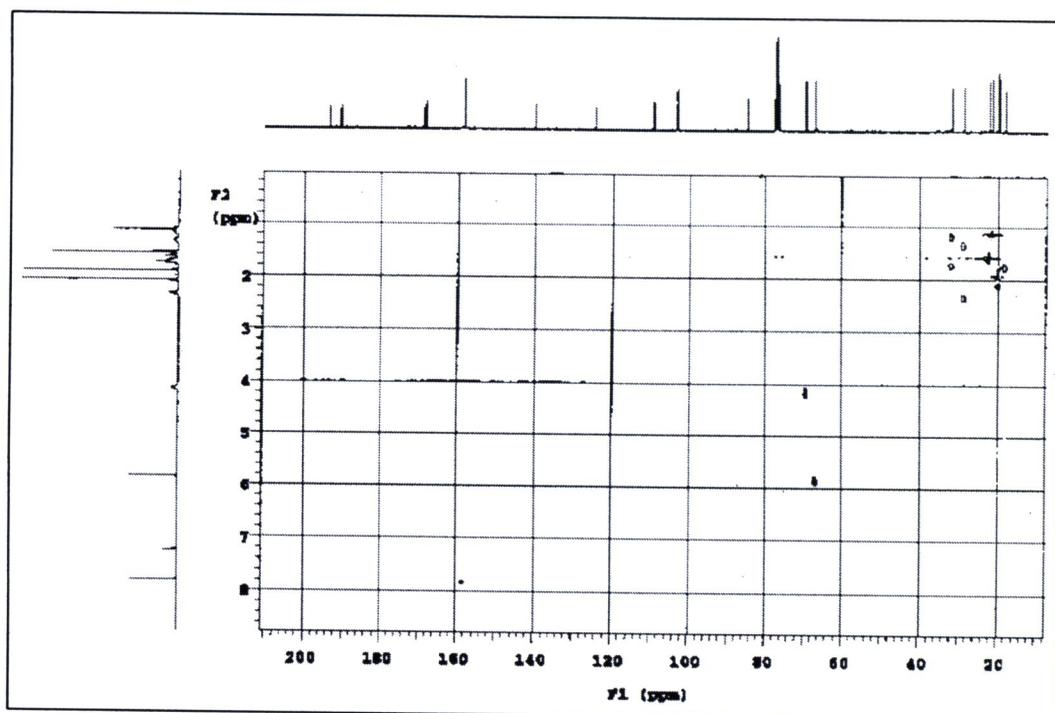


Figure 111 HSQC spectrum of cochliodone D (XV).

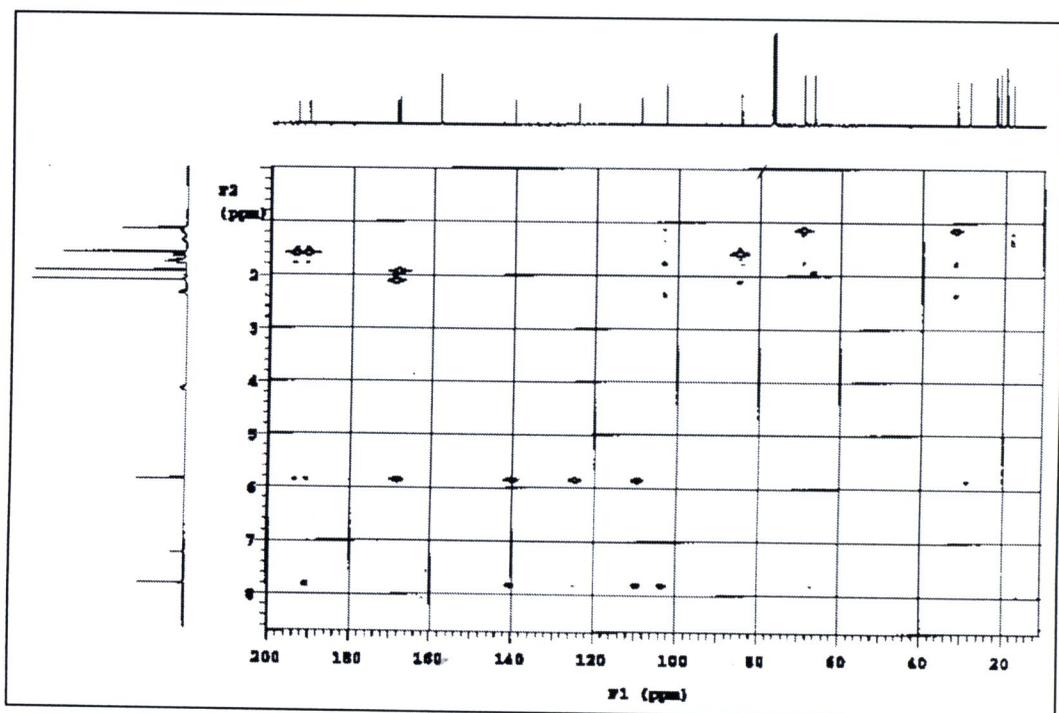


Figure 112 HMBC spectrum of cochliodone D (XV).

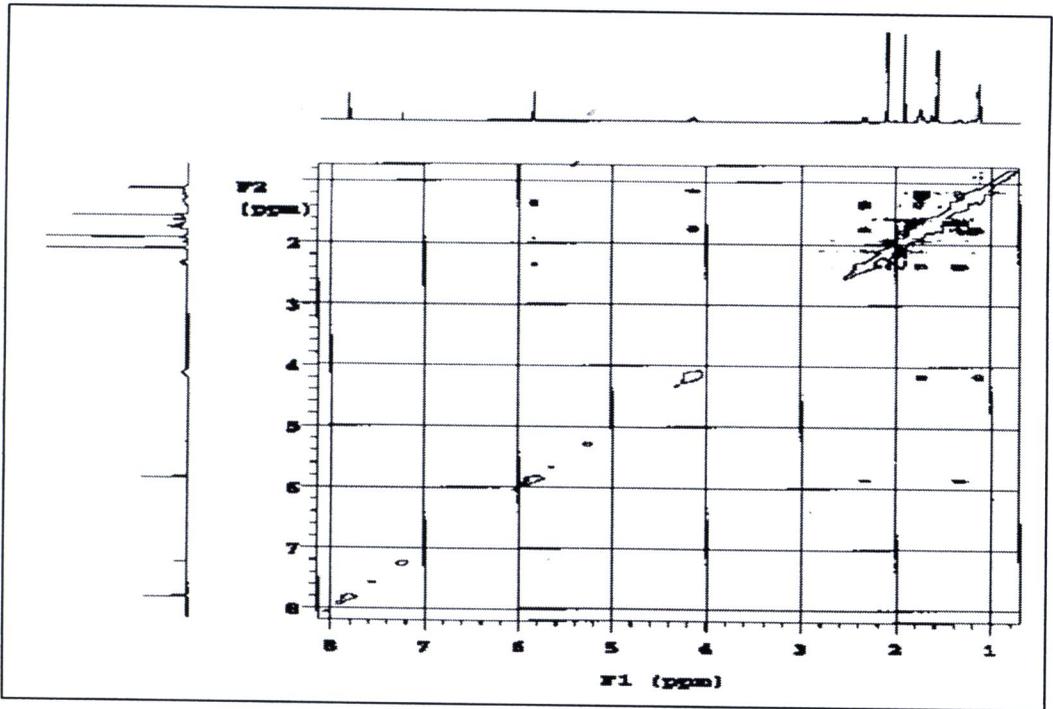


Figure 113 NOESY spectrum of cochlidone D (XV).

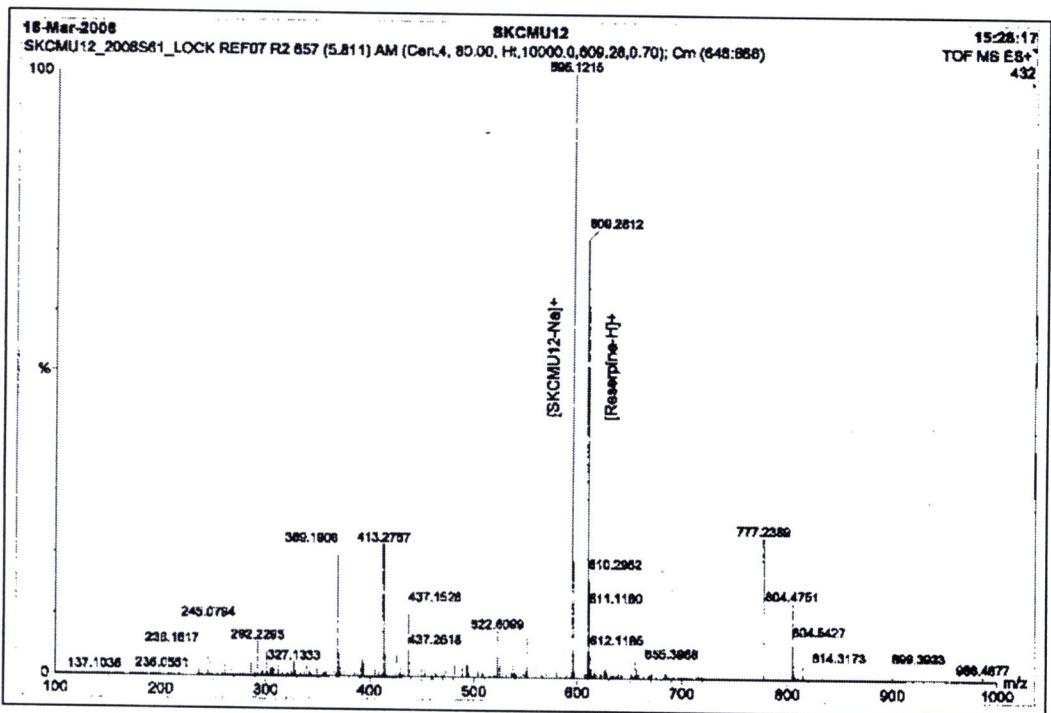


Figure 114 Mass spectrum of Xanthoquinodin A1 (XVI).

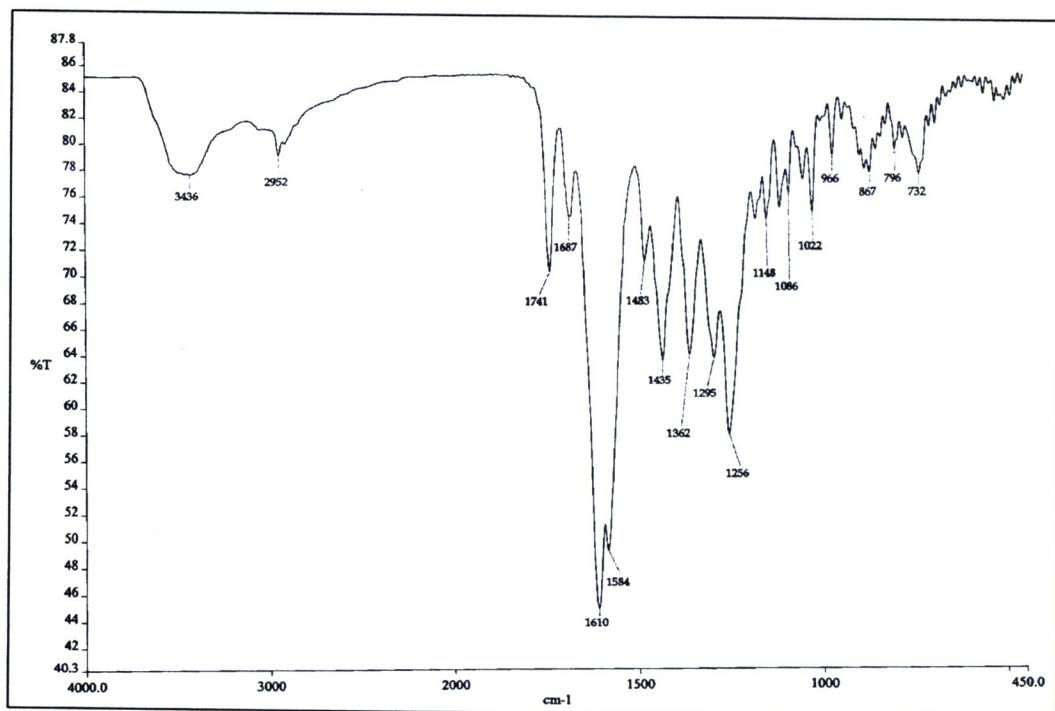


Figure 115 IR spectrum of xanthoquinodin A1 (XVI).

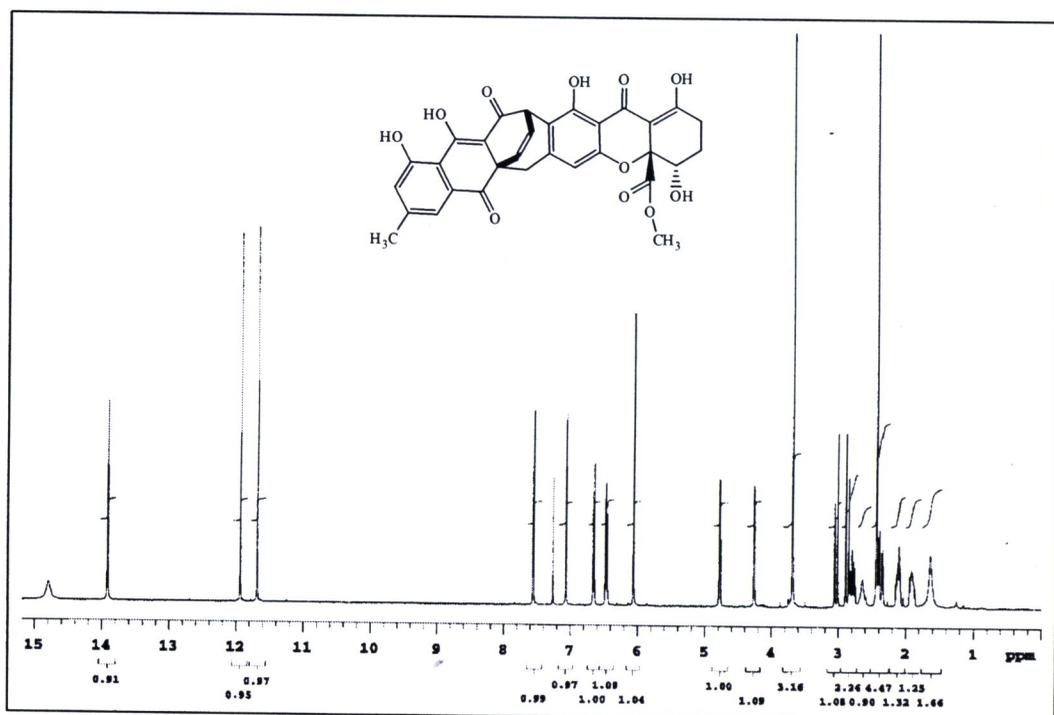


Figure 116 ^1H NMR spectrum of xanthoquinodin A1 (XVI).

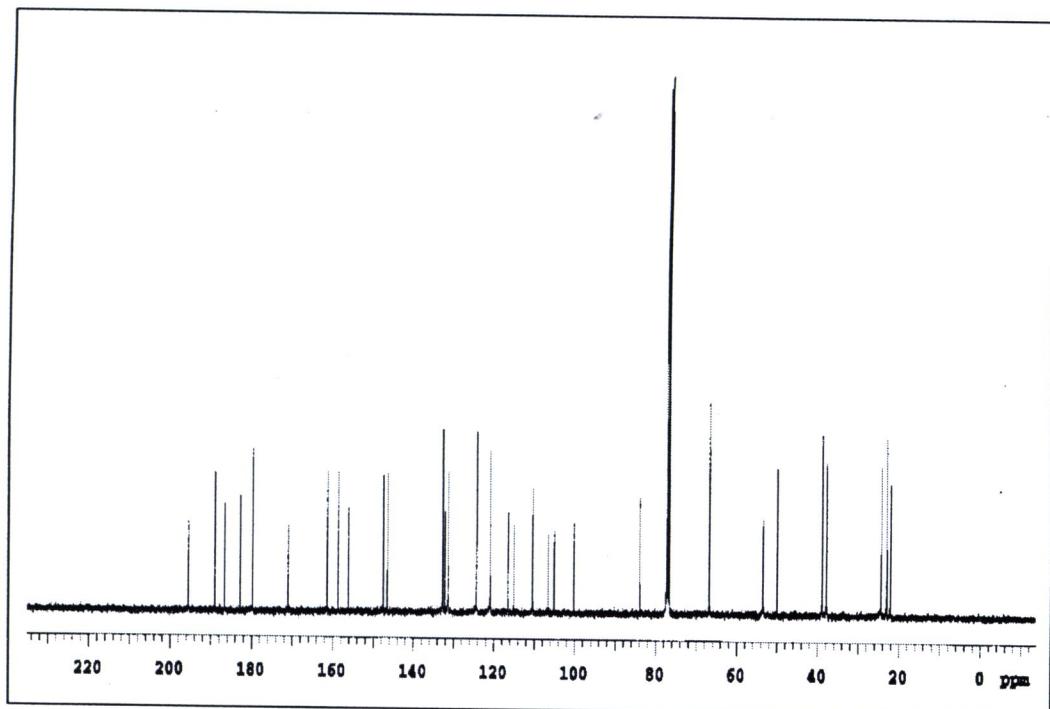


Figure 117 ^{13}C NMR spectrum of xanthoquinodin A1 (XVI).

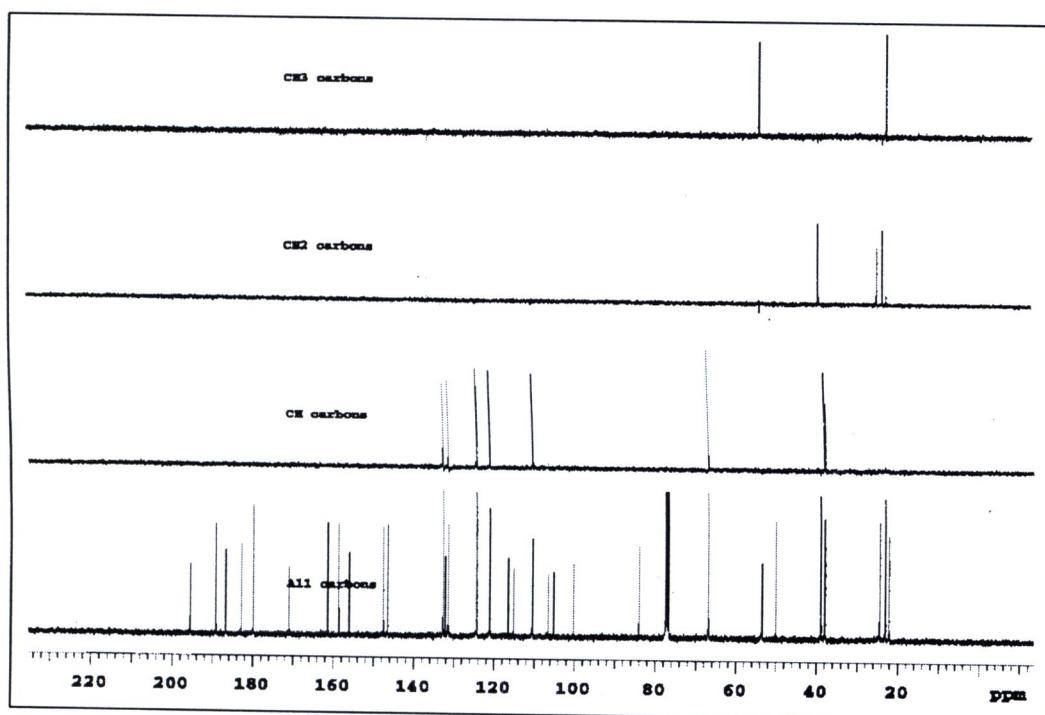


Figure 118 DEPT spectrum of xanthoquinodin A1 (XVI).

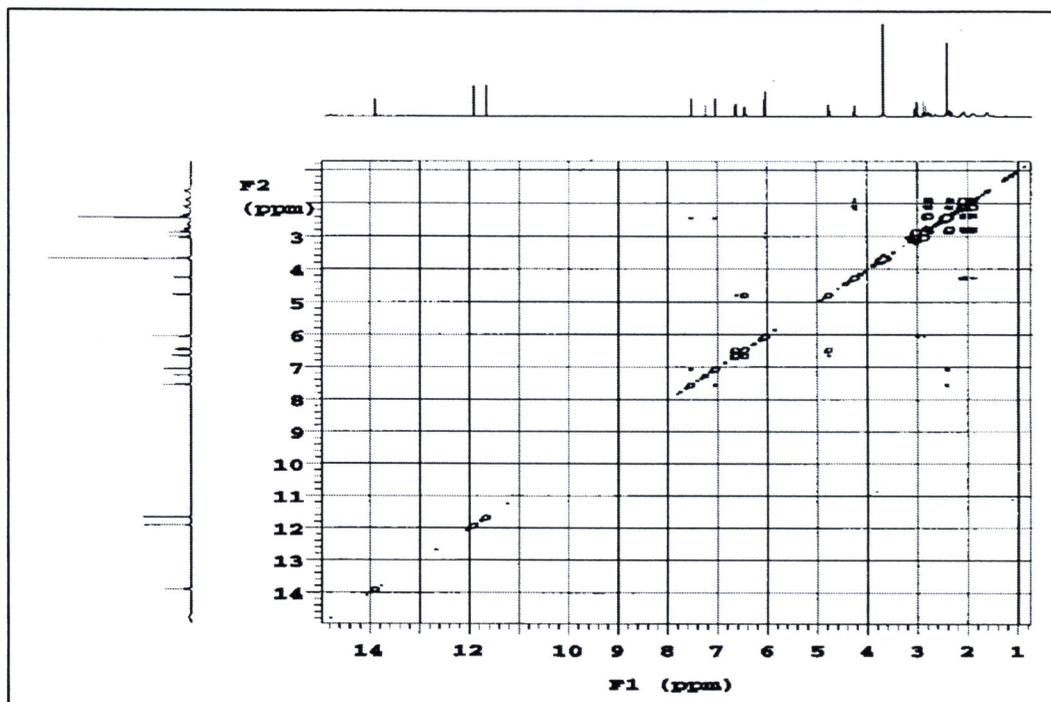


Figure 119 COSY spectrum of xanthoquinodin A1 (XVI).

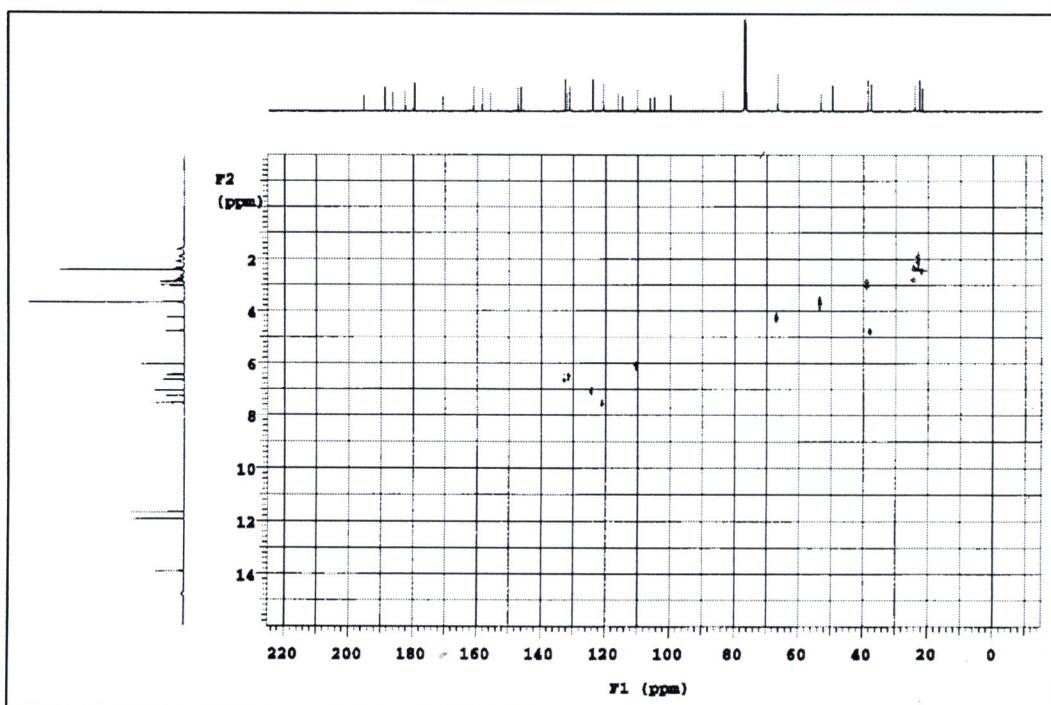


Figure 120 HSQC spectrum of xanthoquinodin A1 (XVI).

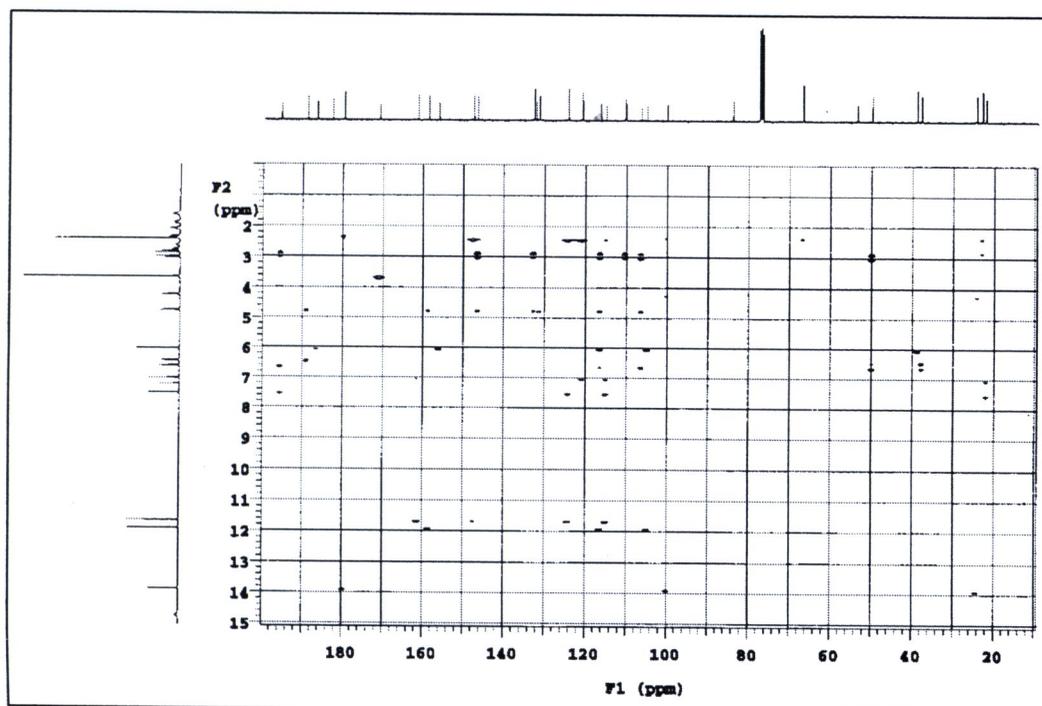


Figure 121 HMBC spectrum of xanthoquinodin A1 (XVI).

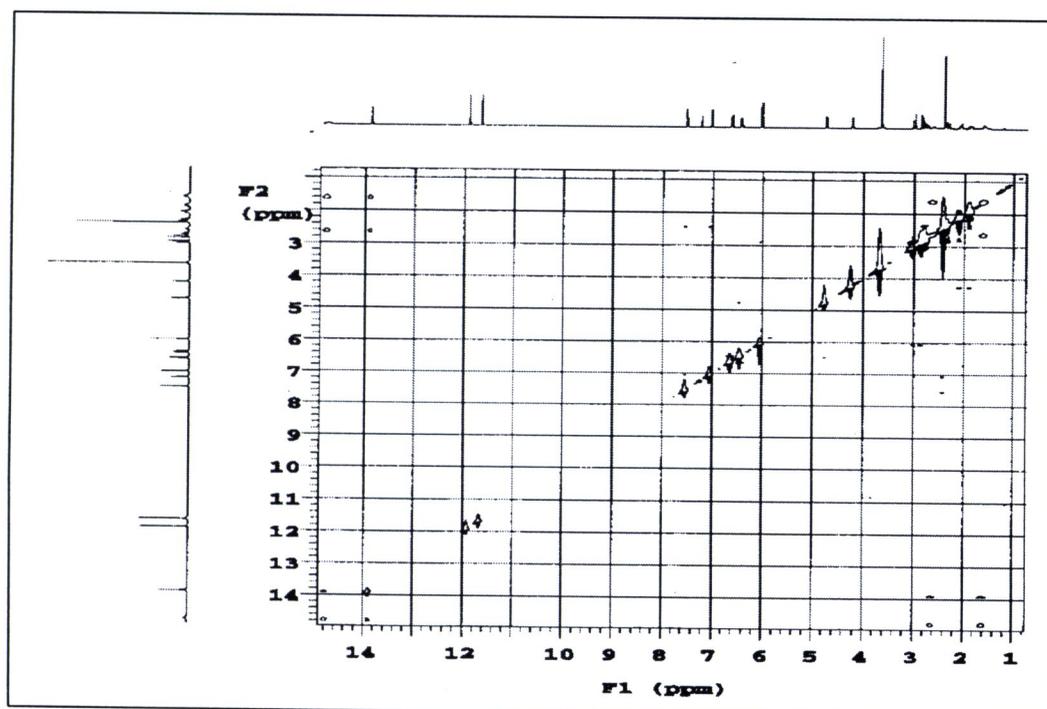


Figure 122 NOESY spectrum of xanthoquinodin A1 (XVI).

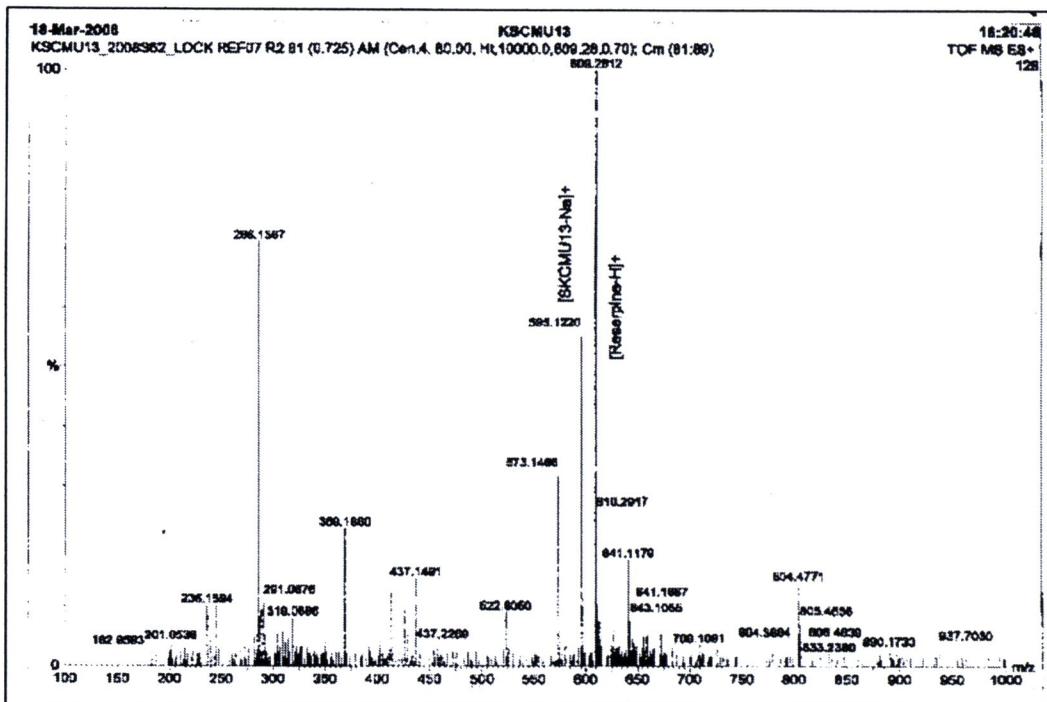


Figure 123 Mass spectrum of xanthoquinodin B2 (XVII).

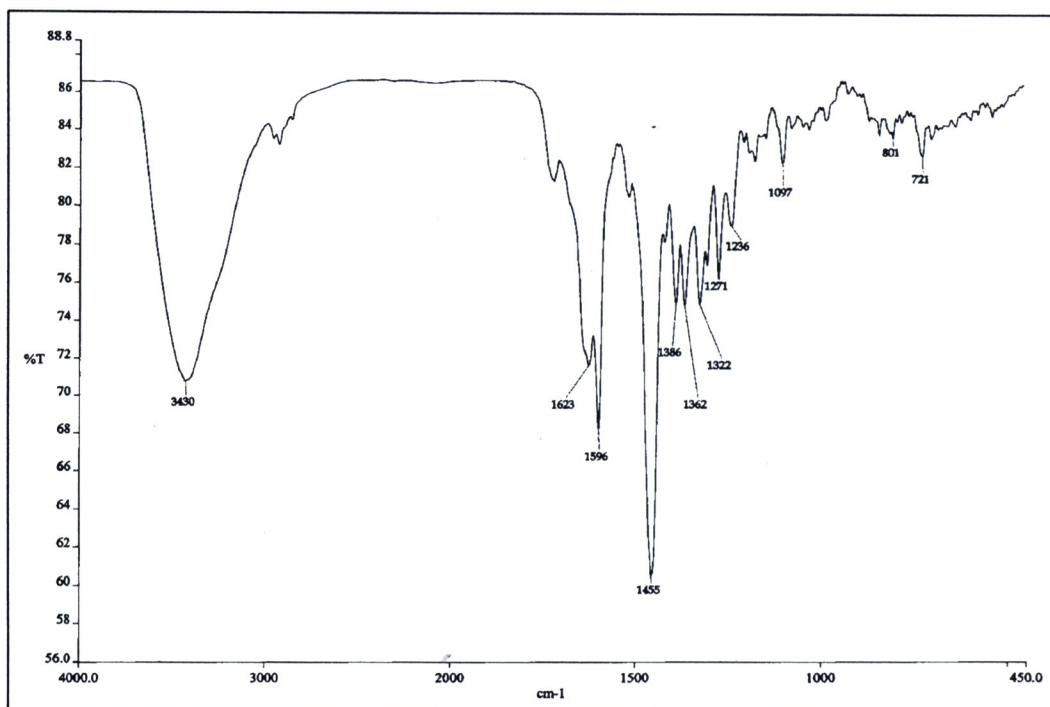


Figure 124 IR spectrum of xanthoquinodin B2 (XVII).

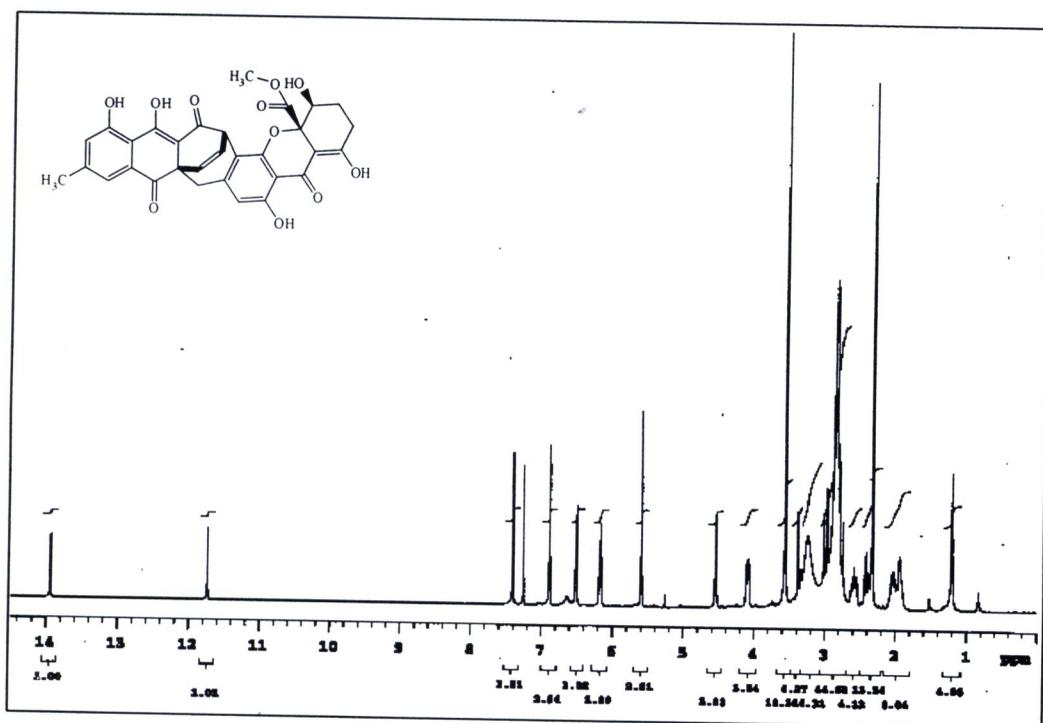


Figure 125 ^1H NMR spectrum of xanthoquinodin B2 (XVII).

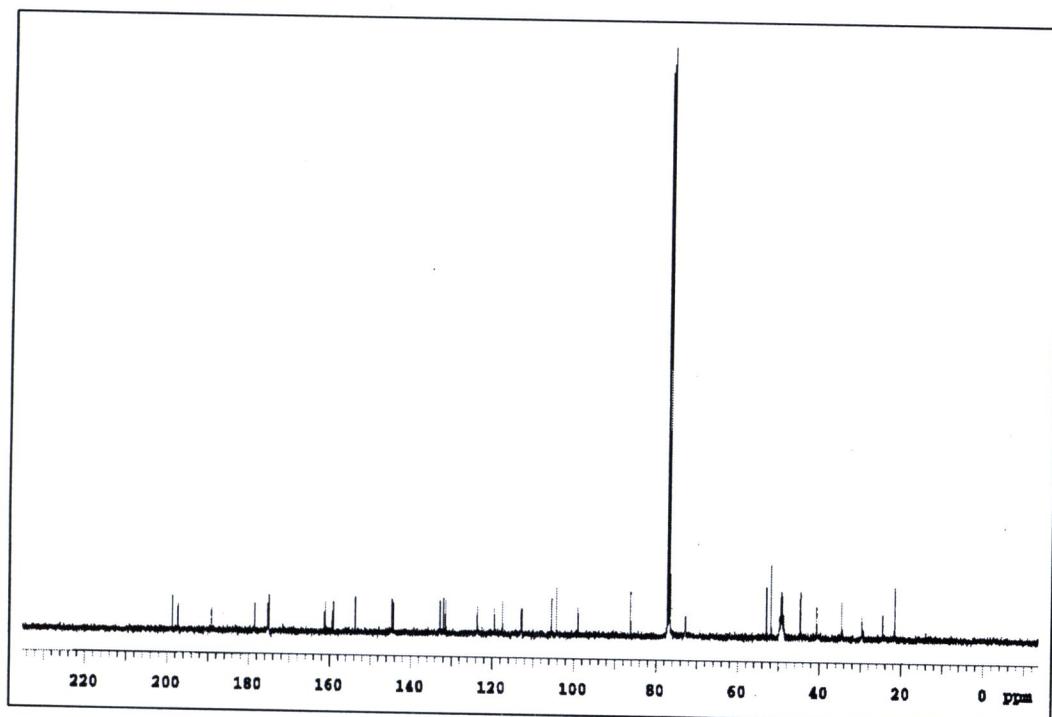


Figure 126 ^{13}C NMR spectrum of xanthoquinodin B2 (XVII).

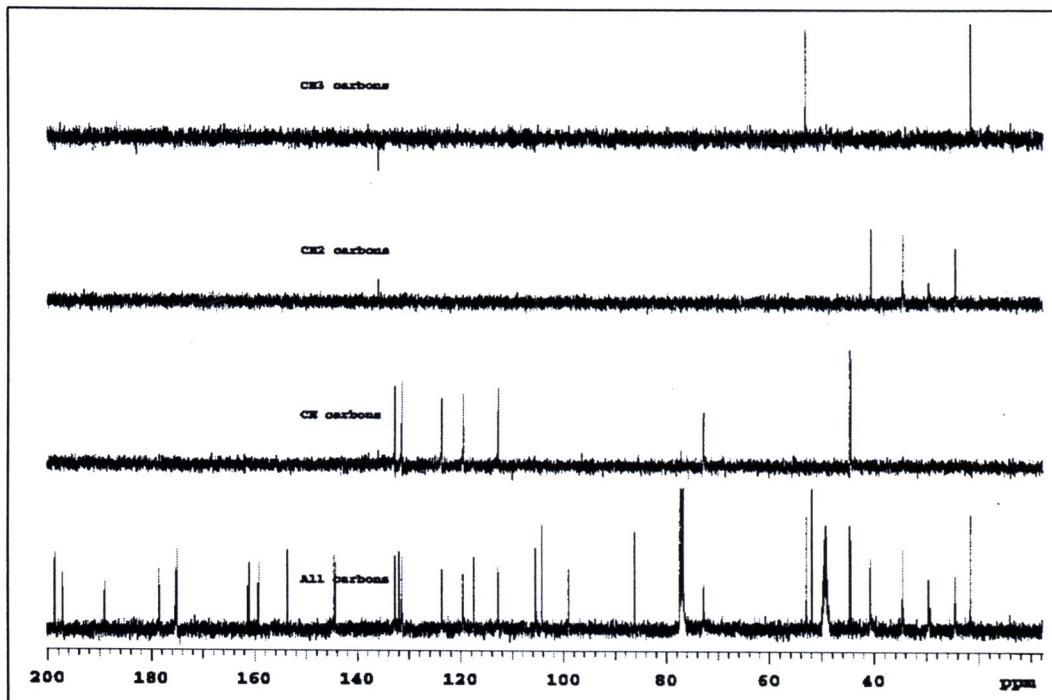


Figure 127 DEPT spectrum of xanthoquinodin B2 (XVII).

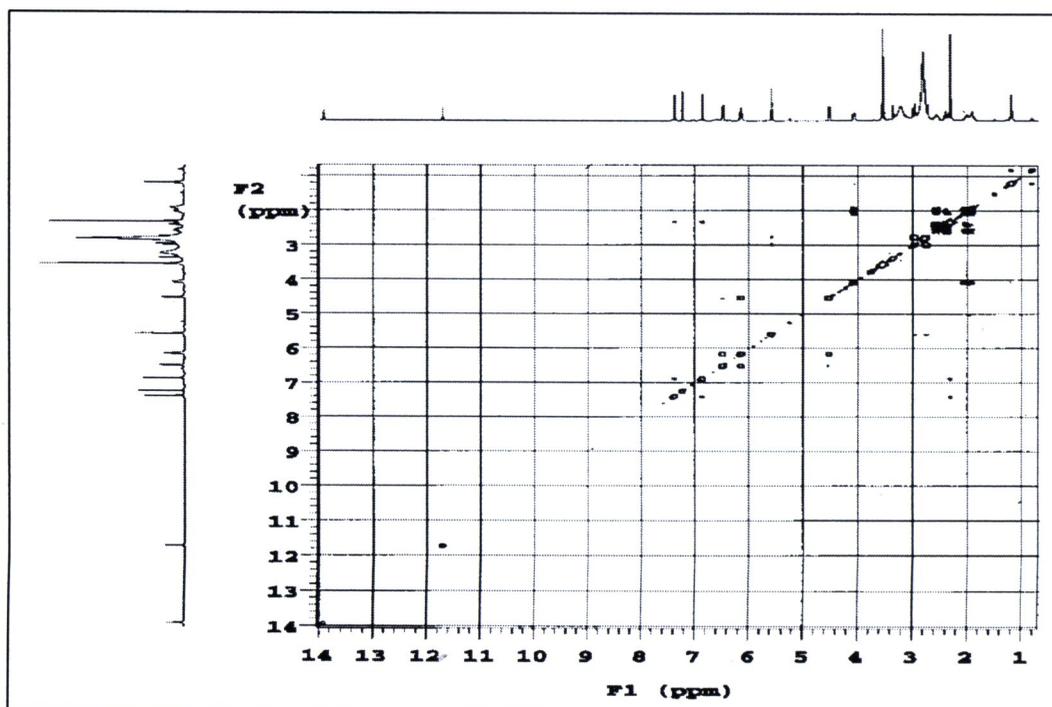


Figure 128 COSY spectrum of xanthoquinodin B2 (XVII).

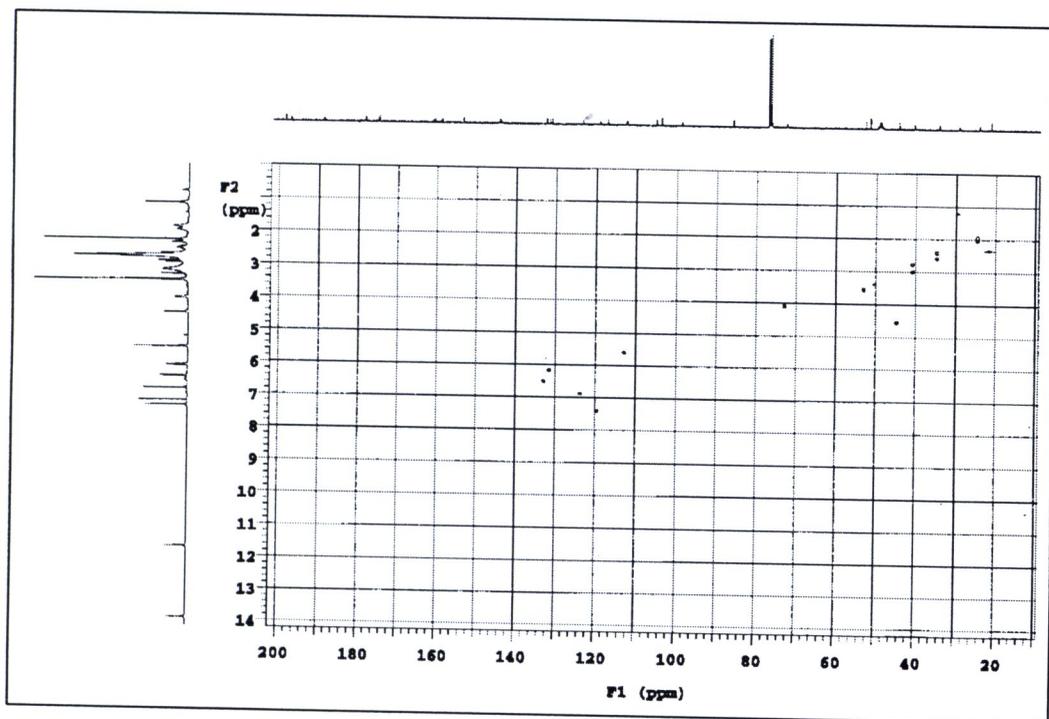


Figure 129 HSQC spectrum of xanthoquinodin B2 (XVII).

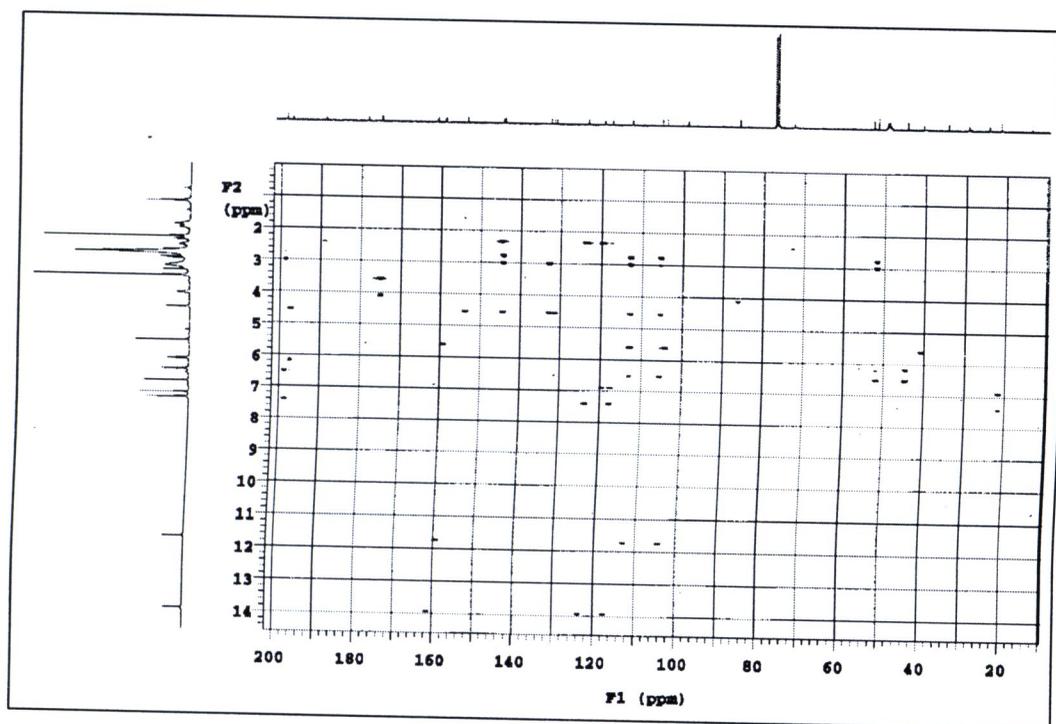


Figure 130 HMBC spectrum of xanthoquinodin B2 (XVII).

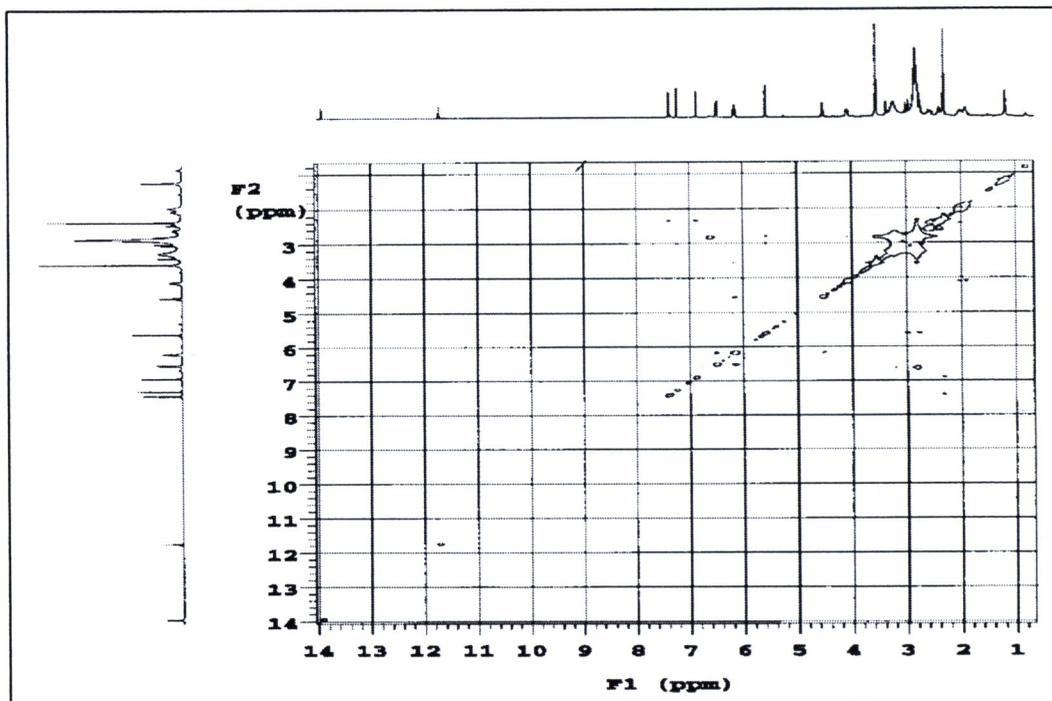


Figure 131 NOESY spectrum of xanthoquinodin B2 (XVII).

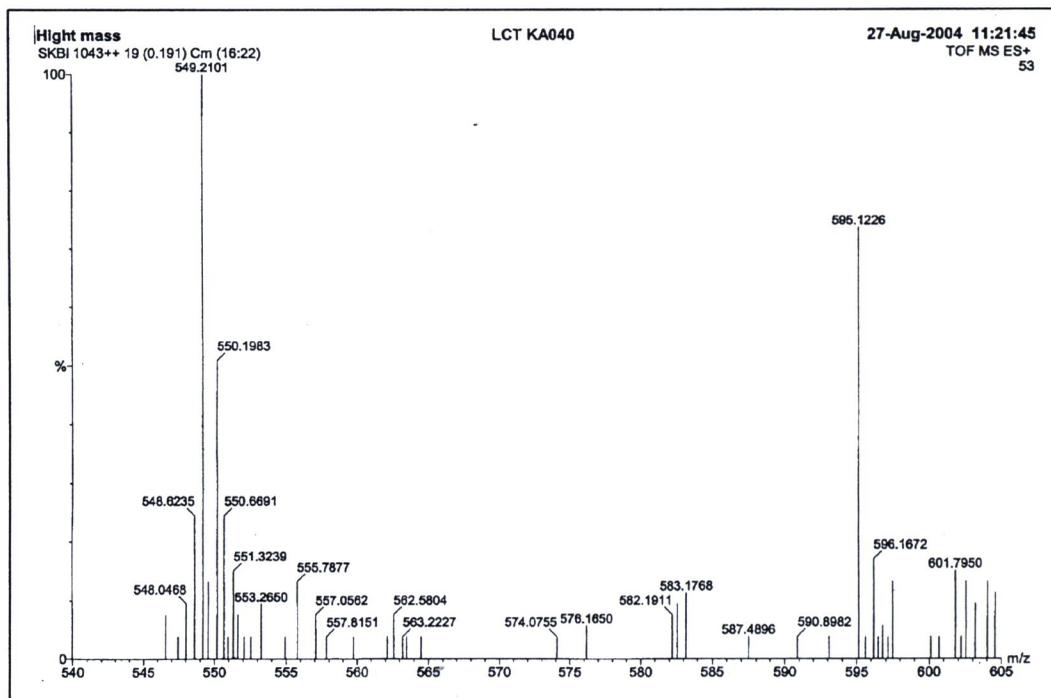


Figure 132 Mass spectrum of XVIII.

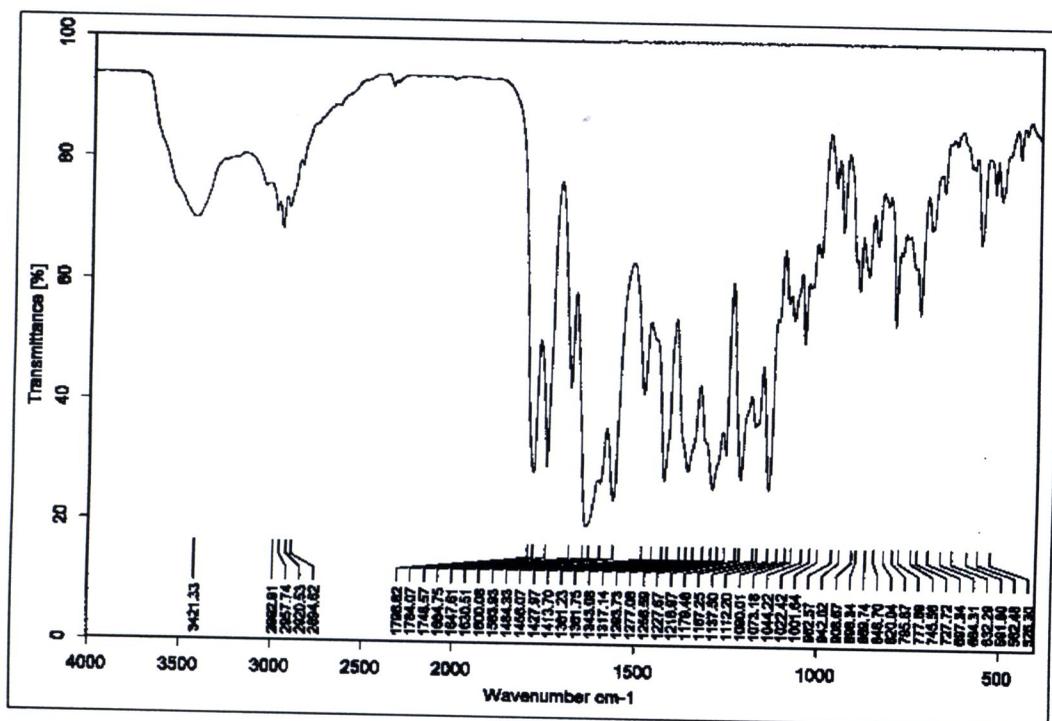
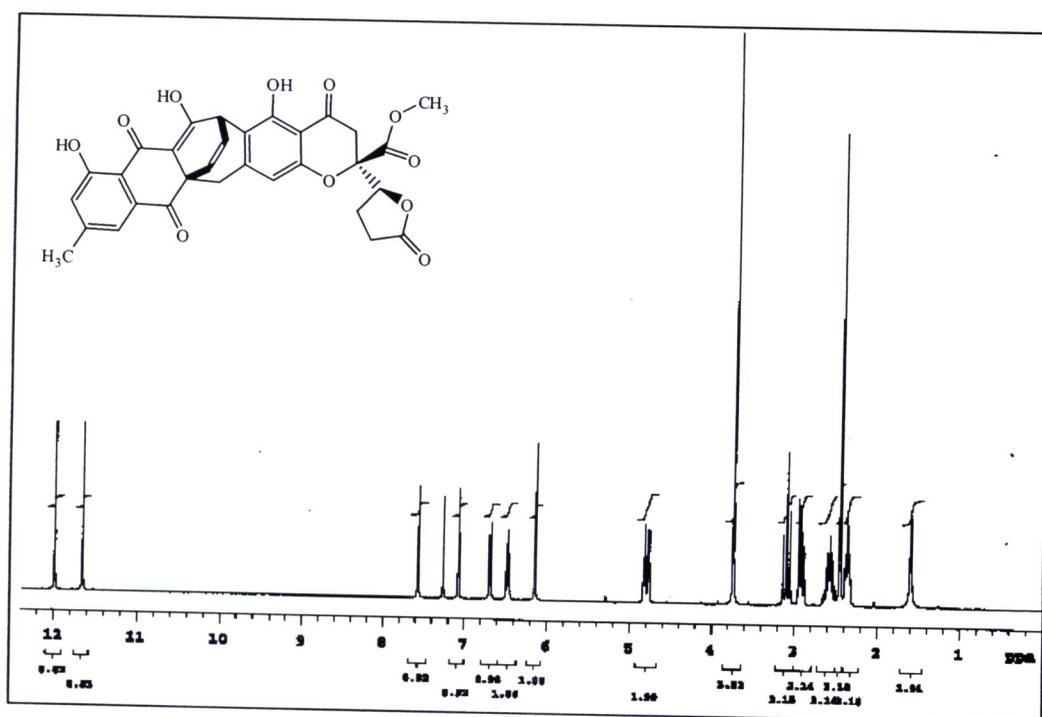


Figure 133 IR spectrum of XVIII.

Figure 134 ¹H NMR spectrum of XVIII.

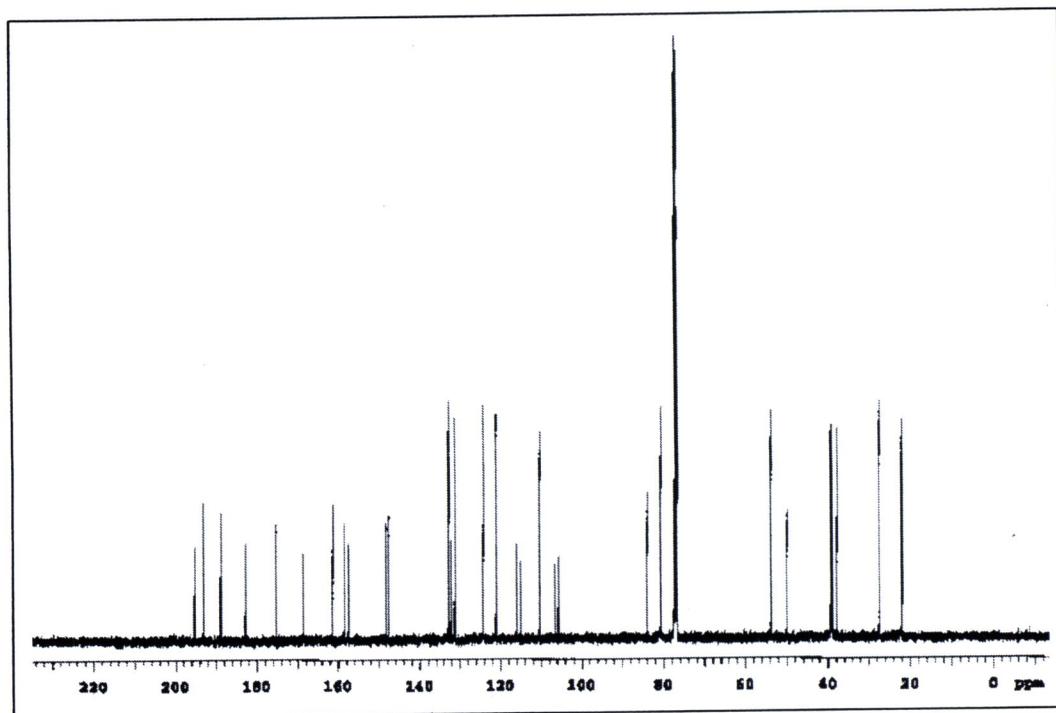


Figure 135 ^{13}C NMR spectrum of XVIII.

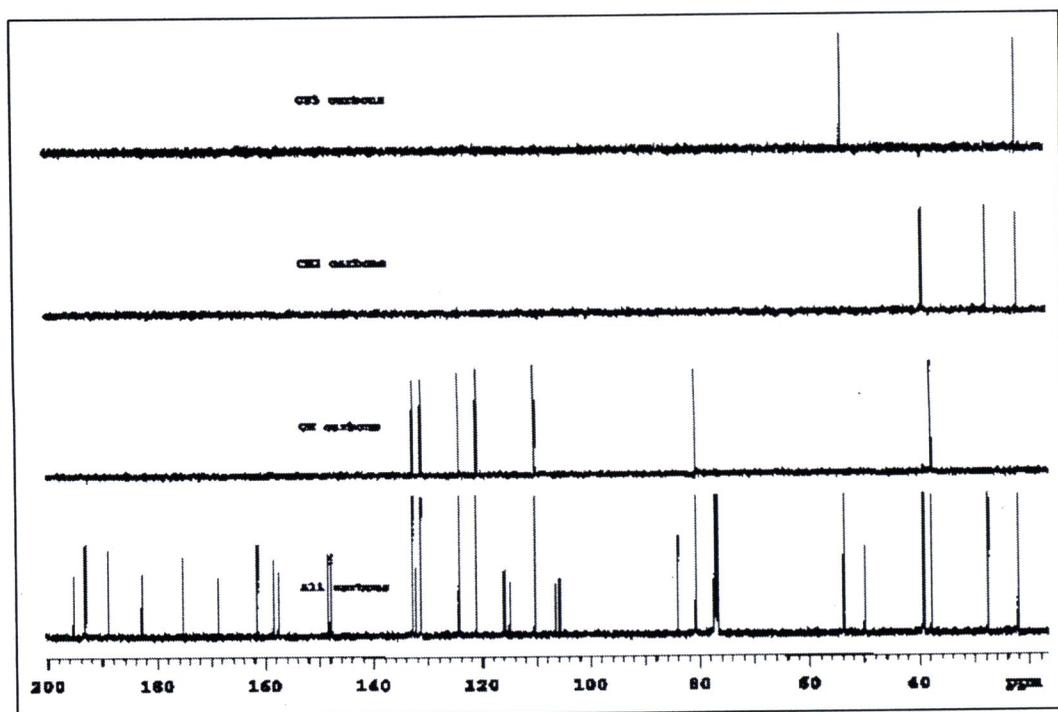


Figure 136 DEPT spectrum of XVIII.

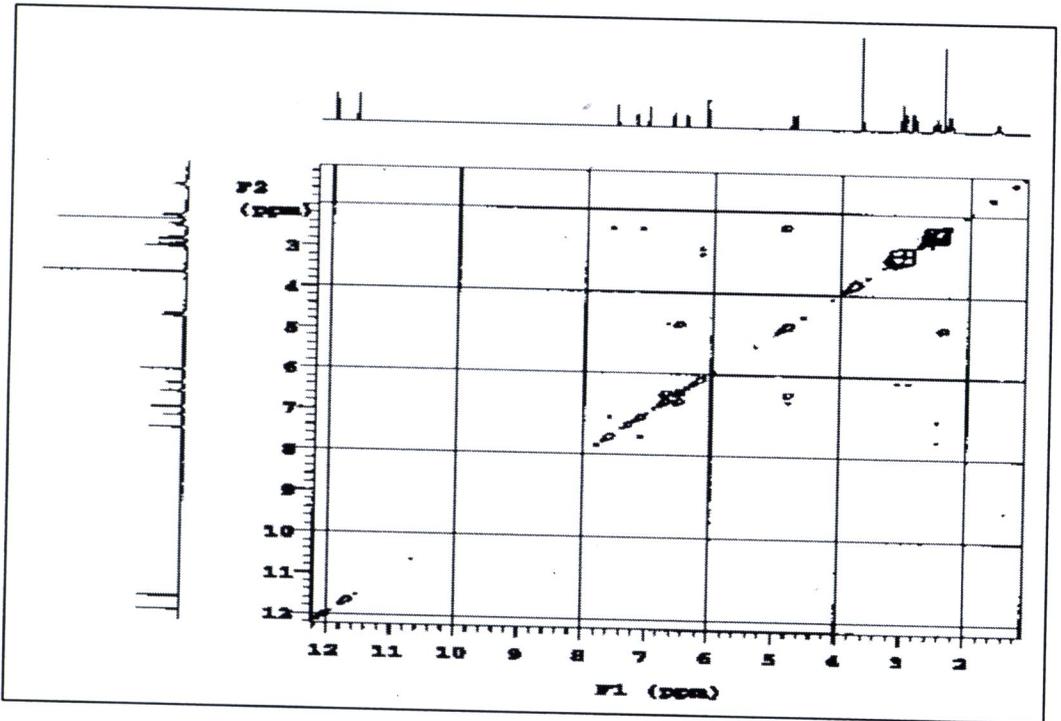


Figure 137 COSY spectrum of XVIII.

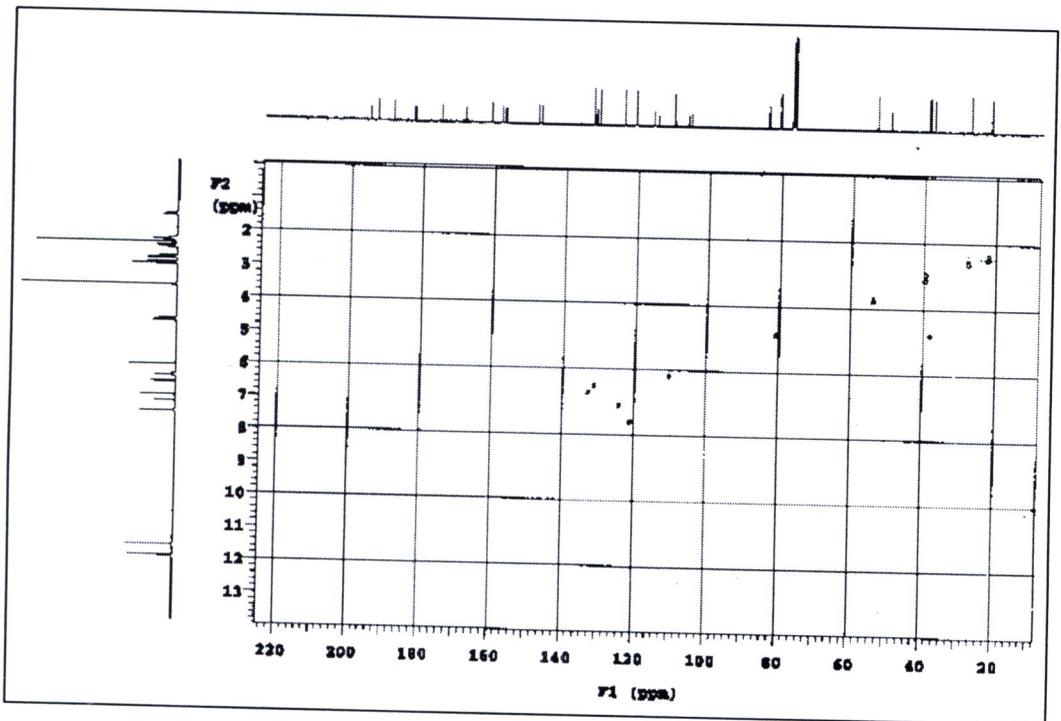


Figure 138 HSQC spectrum of XVIII.

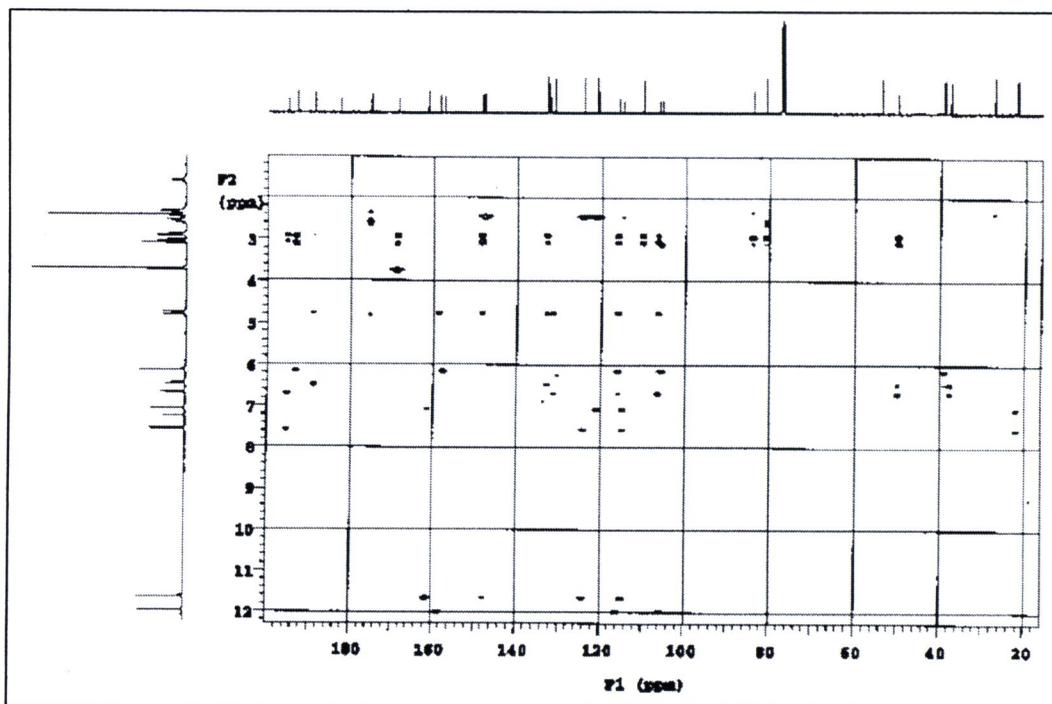


Figure 139 HMBC spectrum of XVIII.

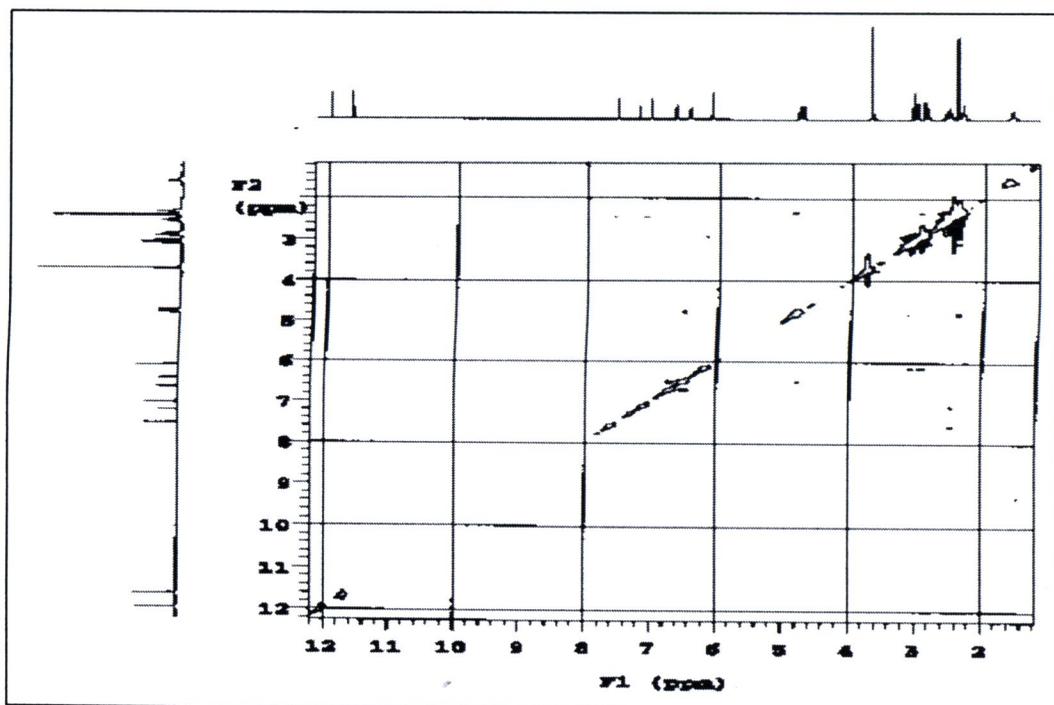


Figure 140 NOESY spectrum of XVIII.

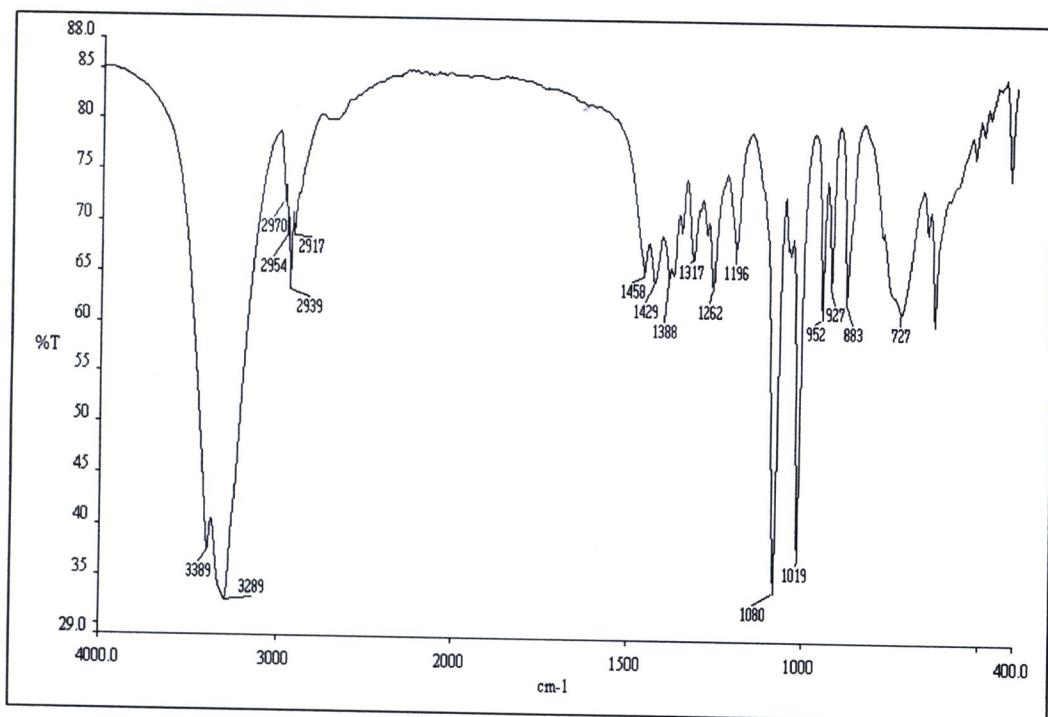


Figure 141 IR spectrum of D-mannitol (XIX).

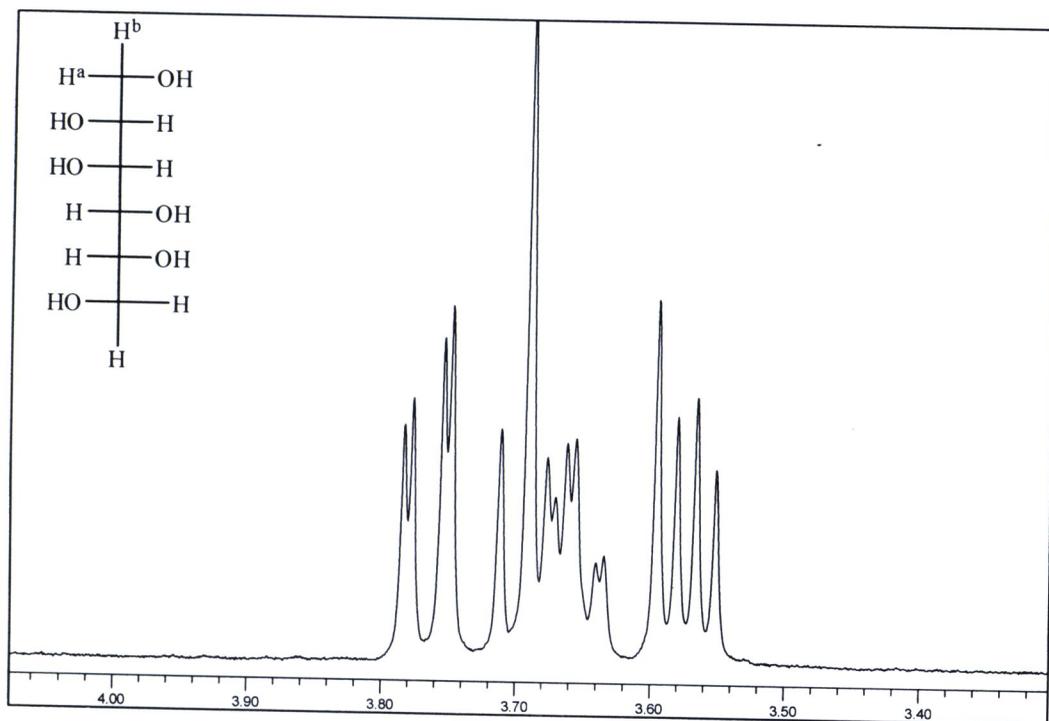


Figure 142 ¹H NMR spectrum of D-mannitol (XIX).

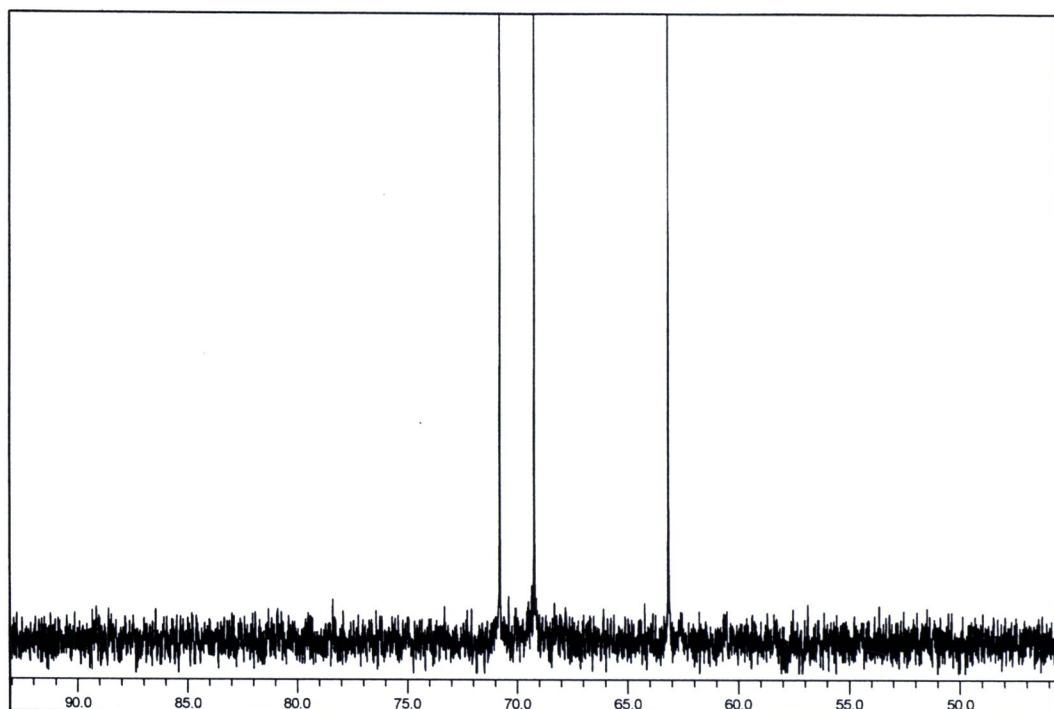


Figure 143 ^{13}C NMR spectrum of D-mannitol (XIX).

RESEARCH PUBLICATIONS

Publication

1. Thohinung, S.; Kanokmedhakul, S.; Kanokmedhakul, K.; Kukongviriyapan, V.; Tusskorn, O; Soyong, K. Cytotoxic 10-(Indol-3-yl)-[13]cytochalasans from the Fungus *Chaetomium elatum* ChE01. *Archives of Pharmacal Research*. **2010**; ...(...): ...-...

Poster Presentation

1. Thohinung, S.; Kanokmedhakul, S.; Kanokmedhakul, K.; Soyong, K. Chemical Constituents from the Fungus *Chaetomium elatum*, at the KMITL International Conference on Integration of Science and Technology for Sustainable Development (ICIST 2007): “Biological Diversity, Food and Agricultural Technology” in April 26-27, **2007**, Faculty of Agricultural Technology, King Mongkut’s Institute of Technology Ladkrabang, Bangkok, Thailand.

IF 1.074 (2008)

Ref.: Ms. No. ARPR-D-10-00019R1

Cytotoxic 10-(Indol-3-yl)-[13]cytochalasans from the Fungus *Chaetomium elatum* ChE01

Archives of Pharmacal Research

Dear Dr. Kanokmedhakul,

I am pleased to tell you that your work has now been accepted for publication in Archives of Pharmacal Research.

It was accepted on 19-04-2010.

Thank you for submitting your work to this journal.

With kind regards

Dongho Lee, Associate Editor

Sang-Geon Kim, Editor in Chief

Mi-Ock Lee, Editor

Archives of Pharmacal Research

Editorial Manager(s) for Archives of Pharmacal Research
Manuscript Draft

Manuscript Number: ARPR-D-10-00019R1

Title: Cytotoxic 10-(indol-3-yl)-[13]cytochalasans from the Fungus *Chaetomium elatum* ChE01

Article Type: Original Article

Corresponding Author: Dr. Somdej Kanokmedhakul, Ph.D.

Corresponding Author's Institution: Khon Kaen University

First Author: Sattit Thohuang, M.Sc.

Order of Authors: Sattit Thohuang, M.Sc.; Somdej Kanokmedhakul, Ph.D.; Kwanjai Kanokmedhakul, Ph.D.; Veerapol Kulkongtanyapan, Ph.D.; Ormanong Tusakorn, M.Sc.; Kasem Soyotong, Ph.D.

Abstract: Nine 10-(indol-3-yl)-[13]cytochalasans, including a new chaetoglobosin V (1), two new natural products, prochaetoglobosin III (2) and prochaetoglobosin IIId (3), six known chaetoglobosins B-D (4-6), F (7), G (8), and neochaetoglobosin D (9), together with two known sterols, 24(R)-28-OH ergosterol (10) and ergosterol (11) were isolated from the fungus *Chaetomium elatum* ChE01. The structures of these compounds were elucidated by spectroscopic methods. Compounds 1-9 showed cytotoxicity against HCT (IC₅₀ 2.54-21.29 μM) and cholangiocarcinoma cell lines (IC₅₀ 3.41-36.95 μM).

Response to Reviewers: Answer to Reviewer

Reviewer #1: Dear Editor,

Manuscript Number: ARPR-D-10-00019

Title: Cytotoxic 10-(indol-3-yl)-[13]cytochalasans from the Fungus *Chaetomium elatum*.

I recommend "accepted after minor revision"

The authors of ARPR-D-10-00019 describe the cytotoxicity of ten cytochalasans, including a new chaetoglobosin V, against cell lines.

General comment:

This is an interesting study that describes cytotoxicity of ten 10-(indol-3-yl)-[13]cytochalasans isolated from a fungal species, *Chaetomium elatum*. This paper is generally clearly written. Thus, the manuscript is acceptable for publication pending adequate revision.

Specific comments:

1) In introduction section (line 35-37), the following sentence "The fungus *Chaetomium elatum* is one of ca. twenty two *Chaetomium* species which have been found in Thailand." should be revised. For example, "About 81 species of *Chaetomium* have been reported worldwide. *Chaetomium elatum* [C. Schmidt & Kuntze] is... found in Thailand."

Detail of this part has been revised.

2) Authors should describe the fungal isolation source (line 35-37): for example, "... soil or leaf of plant.

Detail of this part has been added.

3) Authors should also add a fungal isolate number: for example, "...*Chaetomium elatum* ChE01.

Detail of this part has been added.

4) In IR data, it would be better to mention only main peaks than describe all the peaks in detail.

This has been edited.

5) The (μg/ml) → μM

All unit μg/ml have been changed to μM.

Revised-ARPR-D-10-00019

Noe 10-(indol-3-yl)-[13]cytochalatans, including a new chaetoglobosin V (1), two new natural products, prochaetoglobosin III (2) and prochaetoglobosin III_A (3), six known chaetoglobosins B-D (4-6), F (7), G (8), and isoachaetoglobosin D (9), together with two known sterols, 24(R)-5α,8α-epidioneergosterol-6,22-diene-3β-ol (10) and ergosterol (11) were isolated from the fungus *Chaetomium elatum* CHe01. The structures of these compounds were elucidated by spectroscopic methods. Compounds 1-9 showed cytotoxicity against BCL1 (IC₅₀ 2.54-21.29 μM) and cholangiocarcinoma cell lines (IC₅₀ 3.41-86.95 μM).

Keywords: *Chaetomium*, *Chaetomium elatum*, chaetoglobosin, cytotoxicity, cholangiocarcinoma

Revised-ARPR-D-10-00019

INTRODUCTION

About eighty-one species of *Chaetomium* have been reported worldwide. *Chaetomium elatum* Kunze (Von Arx *et al.*, 1986) is one of ca. twenty two *Chaetomium* species which have been found in Thailand (Soytong, 1991; Petcharat *et al.*, 1991; Pornsuri *et al.*, 2006). Previous investigation on secondary metabolites from the *Chaetomium* species led to the isolation of numerous types of compounds such as benzoquinone derivatives (Brewer *et al.*, 1968), tetra-S-methyl derivatives (Safic *et al.*, 1972), azaphilones (Takahashi *et al.*, 1990; Kanokmedhakul *et al.*, 2006; Phongkerd *et al.*, 2008), bis-azaphilones (Phongkerd *et al.*, 2008) indol-3-yl-[13]cytochalatans, and chaetoglobosin analogs (Sekita *et al.*, 1976; Udagawa *et al.*, 1979; Probst *et al.*, 1981; Sekita *et al.*, 1982; Sekita *et al.*, 1983; Kanokmedhakul *et al.*, 2002; Ding *et al.*, 2006). Moreover, anthraquinone-chromanone (Kanokmedhakul *et al.*, 2002) orsellinic acid, globosoumones (Rashyal *et al.*, 2005) and depasidone (Li *et al.*, 2008; Khunkomkhet *et al.*, 2009) have also been reported. However, no phytochemical investigation of *Chaetomium elatum* has been reported. As part of our work on bioactive constituents from *Chaetomium* species, EtOAc extract of the fungus *C. elatum*, a strain isolated from Thai soil, showed cytotoxicity against BCL1 cell line (IC₅₀ 4.5 μg/mL). We describe herein the isolation, characterization and bioactivity of a new chaetoglobosin V (1), two new natural products, prochaetoglobosin III (2) and prochaetoglobosin III_A (3), together with eight known compounds, chaetoglobosins B-D (4-6), F and G (7 and 8), isoachaetoglobosin D (9), and two ergosterols 10 and 11 (Fig. 1).

MATERIALS AND METHODS

General

Melting points were determined using a Gallenkamp melting point apparatus and were uncorrected. Optical rotations were obtained using a JASCO DIP-1000 digital polarimeter. UV spectra were measured on an Agilent 8453 UV-visible spectrophotometer. IR spectra were taken on a Perkin-Elmer Spectrum One spectrophotometer. NMR spectra were recorded in CDCl₃ and CD₃OD on a Varian Mercury Plus 400 spectrometer, using residual CHCl₃ and CH₃OH as an internal standard. HR-ESI-MS were recorded on a Micromass Q-ToF-2™ spectrometer. Column chromatography and preparative TLC were carried out on silica gel 60 (230-400 mesh) and PF₂₅₄, respectively.

Revised-ARPR-D-10-00019

Fungal material

The fungus was isolated from soil collected in Betong District, Yala Province, Thailand, in June 2005 and was identified by Assoc. Prof. K. Soyong. A voucher specimen (CHe01) was deposited at the Department of Plant Pest Management, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The fungus was cultured in conical flasks (1 L, 70 flasks) with potato dextrose broth (PDB) (250 mL/flask) and incubated in a standing condition at 25-28 °C for 4 weeks. The culture broth was filtered to give a wet mycelial mat and then air-dried at room temperature.

Extraction and Isolation

Air-dried mycelial mat (210 g) of *C. elatum* was ground and extracted successively with hexane (1.5 L × 3), EtOAc (1.5 L × 3), and MeOH (1.5 L × 3) at room temperature. The filtered samples were combined and the solvents were evaporated in vacuo to yield crude hexane (8.4 g, 4.0 %), EtOAc (21.7 g, 10.3 %), and MeOH (28.5 g, 13.6 %) extracts, respectively. The hexane extract (8.4 g) was subjected to silica gel flash column chromatography (FCC), eluted with a gradient system of hexane, hexane-EtOAc, EtOAc-MeOH, and followed by MeOH. Each fraction (100 mL) was monitored by TLC fractions and similar TLC patterns were combined to yield seven further fractions (HF₁-HF₇). Fraction HF₁ (40-50% EtOAc-hexane) was chromatographed on silica gel FCC, eluted with a gradient system of hexane-EtOAc to afford a white solid of 10 (129.0 mg) and 11 (23.6 mg).

EtOAc (50 mL) was added to the EtOAc extract (21.7 g) to give a white solid of chaetoglobosin C (5) (2.4 g, mp 238-239 °C, [α]_D²⁰ -80 (c 0.10, MeOH)). The filtrate was evaporated to yield a residue which was subjected to silica gel FCC, eluted with the same gradient system as the hexane extract above to give seven fractions, EF₁-EF₇. Fraction EF₂ was chromatographed on silica gel FCC, eluted with a gradient system of hexane-EtOAc to afford an additional amount of 10 (212.1 mg) and 11 (28.5 mg). Fraction EF₃ was separated over silica gel FCC, eluted with a gradient system of hexane-EtOAc and MeOH to yield three subfractions, EF₃₁-EF₃₃. Subfraction EF₃₁ was washed with CH₂Cl₂-MeOH (90:10) and recrystallized from CH₂Cl₂-MeOH to give crystals of prochaetoglobosin III_A (3) (43.4 mg, mp 240-241 °C, [α]_D²⁰ -45 (c 0.12, MeOH)). The precipitate in fraction EF₃₂ was filtered out and recrystallized from CH₂Cl₂-MeOH to give an additional amount of 5 (37.2 mg). Subfraction EF₃₃ was separated over silica gel FCC, eluted with a gradient system of

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EtOAc-hexane to give seven subfractions, EF₃₃₁-EF₃₃₇. Subfraction EF₃₃₁ was further separated by preparative TLC using CH₂Cl₂-MeOH (90:10) as eluent (developed a 21) to give a pale yellow solid of prochaetoglobosin III (2) (21.0 mg, mp 137-138 °C, [α]_D²⁰ -282 (c 0.10, MeOH)) and a white solid of 1 (10.9 mg), respectively. Fraction EF₃₃₂ was chromatographed on a silica gel FCC, eluted with a gradient system of hexane-EtOAc and EtOAc-MeOH to give eight subfractions, EF₃₃₂₁-EF₃₃₂₈. The white precipitate in subfraction EF₃₃₂₁ was filtered out to give an additional amount of 5 (14.2 mg). Subfraction EF₃₃₂₂ was recrystallized from CH₂Cl₂-MeOH to yield a pale yellow solid of chaetoglobosin B (4) (204.2 mg, mp 187-189 °C, [α]_D²⁰ -242 (c 0.10, MeOH)). Subfraction EF₃₃₂₃ was further recrystallized from CH₂Cl₂-MeOH to give isoachaetoglobosin D (9) (17.0 mg, mp 261-262 °C, [α]_D²⁰ +47 (c 0.13, MeOH)). Subfraction EF₃₃₂₄ was then separated over silica gel FCC, eluted with a gradient system of CH₂Cl₂-MeOH to afford four subfractions, EF₃₃₂₄₁-EF₃₃₂₄₄. Subfraction EF₃₃₂₄₁ yielded an additional amount of 5 (16.8 mg). The white subfraction EF₃₃₂₄₂ gave a white solid which recrystallized from CH₂Cl₂-MeOH to give chaetoglobosin G (8) (9.3 mg, mp 249-251 °C, [α]_D²⁰ -148 (c 0.13, MeOH)). The solid in subfraction EF₃₃₂₄₃ was filtered out and recrystallized from CH₂Cl₂-MeOH to give an additional amount of 9 (50.1 mg). Subfraction EF₃₃₂₄₄ was recrystallized from CH₂Cl₂-MeOH to give pale yellow crystals of chaetoglobosin D (6) (43.8 mg, mp 215-216 °C, [α]_D²⁰ -346 (c 0.10, MeOH)). Subfraction EF₃₃₂₅ was chromatographed on silica gel, eluted with a gradient system of hexane-EtOAc to give a white solid of chaetoglobosin F (7) (119.6 mg, mp 173-175 °C, [α]_D²⁰ -124 (c 0.14, MeOH)).

The MeOH extract (28.5 g) was chromatographed on a silica gel FCC, eluted with a gradient system of CH₂Cl₂-MeOH to give five fractions, MF₁-MF₅. Fraction MF₂ was recrystallized from CH₂Cl₂-hexane to afford an additional amount of 10 (3.9 mg). The precipitate in fraction MF₃ was filtered out and recrystallized from CH₂Cl₂-MeOH to yield an additional amount of 5 (1.64 g).

Chaetoglobosin V (1): A white solid, mp 240-241 °C, [α]_D²⁰ -28 (c 0.12, MeOH); UV_{max} (MeOH) nm (log ε) 222 (4.48), 280 (3.72), 290 (3.62); IR (KBr) ν_{max} 3522, 3329, 2951, 1745, 1704, 1676, 1657, 1616, 1259, 1233, 1046 cm⁻¹; HRESITOFMS m/z 521.2780 [M+Na]⁺ (Calcd for C₃₁H₄₀N₂O₅ + Na, 521.2780); ¹H- and ¹³C-NMR data, see Table 1.

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Cytotoxicity assay

Cytotoxic assay against human breast cancer (BC1) cell line was performed employing the colorimetric method described by Skchan P., *et al.*²⁶. The reference substance was elliposine (Table 2). Cholangiocarcinoma (CCA) cultured cell lines established in the Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, including KKKU-100, and KKKU-OCA17 derived from human intrahepatic cholangiocarcinoma tissues with histological types of poorly differentiated, and well differentiated adenocarcinoma, respectively (Burarat *et al.*, 2007; Prawn *et al.*, 2009). The CCA cells were routinely cultured in Ham's F12 media supplemented with 4 mM L-glutamine, 1 mM sodium pyruvate, 12.5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES, pH 7.3), 100 U/ml penicillin, 100 µg/ml streptomycin sulfate, and 10% fetal calf serum. The media was renewed every 3 days, trypsinized with 0.25% trypsin-EDTA, and subcultured in the same media. The assay against cholangiocarcinoma cell line was performed employing the method described by Voigt (2005). The reference substance was 5-fluorouracil (Table 2).

RESULTS AND DISCUSSION

Nine chactoglobosins and two sterols were isolated as a white solid from hexane, EtOAc, and MeOH extracts of dried mycelial mat of *C. sitatum* using the combination of silica gel column chromatography and preparative TLC. Structures of the known compounds were identified by physical and spectroscopic data measurements (IR, ¹H- and ¹³C-NMR, 2D NMR, and MS) and by comparing the data obtained with published values, as chactoglobosins B-D (4-6), F (7), G (8) (Sekita *et al.*, 1982; Sekita *et al.*, 1983), chactoglobosin D (9) (Sekita *et al.*, 1982), 24(8)-5a,8a-epidioxycyclosta-2,6-diene-3β-ol (10) (Bok *et al.*, 1999), and ergosterol (11) (Oh *et al.*, 1998). Prochactoglobosin IIIa (2) and prochactoglobosin IIIa (3) have been isolated from *Chaetomium subaffine* after treating with a specific oxidative enzyme, cytochrome P450 inhibitors (Oikawa *et al.*, 1991). Therefore, this is the first report of 2 and 3 as new naturally occurring compounds.

Compound 1 was obtained as a white solid and showed a molecular ion in the HRESITOFMS at *m/z*: 521.2780 [M+Na]⁺, indicating a molecular formula C₃₃H₄₆N₂O.

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The IR spectrum showed the absorption bands of NH and OH (3264-3522 cm⁻¹), ketone carbonyl (1704 cm⁻¹), amide (1657 cm⁻¹), and aromatic (1616 cm⁻¹) groups. The UV spectrum of 1 indicated the presence of an α,β-unsaturated ketone and indole chromophore with λ_{max} of 222, 280, and 290 nm (Silvertown *et al.*, 1976). ¹³C-NMR and DEPT spectra indicated 32 carbons attributable to two carbonyl, six sp³ quaternary, ten sp² methine, one sp³ quaternary, five sp³ methine, four sp³ methylene, and four methyl carbons. The ¹H-NMR spectrum displayed five low-field signals of an indole unit at δ 6.94 (d, H-2), 6.97 (t, J = 7.4 Hz, H-5'), 7.07 (t, J = 7.5 Hz, H-6'), 7.29 (d, J = 8.2 Hz, H-7'), and 7.44 (d, J = 7.8 Hz, H-4'). Four signals of *trans*-double bond coupled protons showed at δ 5.16 (ddd, J = 2.7, 10.1, 15.2 Hz, H-14), 5.92 (ddd, J = 1.6, 9.7, 15.2 Hz, H-13), 6.81 (ddd, J = 5.5, 9.7, 15.2 Hz, H-21) and 6.33 (d, J = 15.2 Hz, H-22). The olefinic proton appeared at 4.60 (d, J = 9.7 Hz, H-17), while four methyl groups appeared at 0.85 (d, J = 6.6 Hz, H-24), 1.34 (s, H-11), 1.50 (s, H-25), and 1.60 (s, H-12). Four sp³ methine protons showed at δ 3.46 (t, J = 7.6 Hz, H-3), 2.90 (tvs, H-4), 3.78 (d, J = 8.5 Hz, H-7) and 2.43 (m, H-16). The structure was then confirmed by analysis of the 2D NMR spectra, including COSY, HSQC, HMBC, and NOESY. Therefore, three partial structural units were assigned by analysis of the COSY spectrum which showed correlations between H-10→H-3→H-4, H-7→H-8→H-13→H-14→H-15→H-16→H-24 and H-17→H-19 (allylic)→H-20→H-21→H-22, and H-4→H-5→H-6→H-7 (Fig. 2). Interpretation of the HMBC spectrum of 1 (Table 1 and Fig. 2) led to the assembly of these three proton networks and the remaining hydrogen and non-protonated carbons, to complete the whole structure of 1. The relative configuration of compound 1 was assigned on the basis of the coupling constants and the NOESY correlations of these protons. The coupling constant (8.5 Hz) of H-7 to H-8 implied that H-8 is arranged axial and *trans* to H-8. The configuration of double bonds on the C-13, C-14 and C-21, C-22 were found to be *E* on the basis of their coupling constants (*J*_{13,14} and *J*_{21,22} = 15.2 Hz). The lack of correlation between H-17 and methyl protons at C-25 in the NOESY spectrum indicated the *E* configuration of C-17 alkene. The NOESY spectrum showed correlations between H-4 and H-8, indicating that H-4 was an axial and *cis* to H-8. Finally, the NOESY correlations between H-17 and H-15, H-16, and H-19; H-20 and H-15, and H-19 revealed that the conformation of the macrocyclic ring of 1 was the same as that reported for prochactoglobosin I (Oikawa *et al.*, 1991). On the basis of the above data, 1 was deduced as a new 10-(indol-3-yl)-[13]cytchalasan which has been named chactoglobosin V.

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The isolated compounds were evaluated for their cytotoxicity against human breast cancer cell (BC1) at the Bioss Research Facility of the National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA, Thailand, and the cytotoxicity against cholangiocarcinoma cell lines was tested at the Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

All compounds showed cytotoxicity against BC1 cell lines with IC₅₀ values in the range 2.54-21.29 µM (Table 2). Among these, 3 and 4 exhibited strong cytotoxicity to BC1 cell line with IC₅₀ values of 2.54 and 3.03 µM, respectively. Compounds 1-9 were also tested against two different cholangiocarcinoma cell types, KKKU-100 cells which are resistant to chemotherapeutic agent, 5-fluorouracil (5-FU), and KKKU-OCA17 cells which are sensitive to the 5-FU. It is apparent that chactoglobosin D (8) was the most active against both cell types with IC₅₀ values of 3.41 and 12.12 µM, respectively, while isochoactoglobosin D (9) was active against KKKU-100 cells with IC₅₀ value of 7.41 µM (Table 2).

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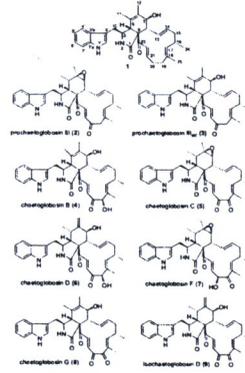
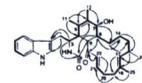


Fig. 1. Structures of compounds 1-9



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Fig. 2. Key COSY correlations (bold lines) and selected HMBC correlations (H—C) of chaetoglobosin V (1).

Table 1. ¹H- and ¹³C-NMR Spectral Data (δ, ppm) in CD₃OD+CDCl₃

Position	¹ H	¹³ C	HMBC (H—C)	NOESY
1	-	175.2 ^a	-	-
2	8.47	-	-	-
3	3.46 (7.6 ^b)	58.4	C-1, 3, 4, 5, 9, 3'	H-4, 10, 12, 4'
4	2.90 br	48.1	C-6	H-3, 8, 10, 12, 15,
5	-	126.0	-	22
6	-	133.5	-	-
7	3.78 d (8.5)	48.1	-	H-11, 13, 15
8	2.24 m	50.4	C-1, 21, 4, 3', 9, 13,	H-4, 11, 14, 24
9	-	60.8	16	-
10	2.87 dd (7.6, 14.2)	31.6	C-3, 2', 3', 3x	H-3, 10
11	1.34	141.4	C-4, 4', 5, 6, 12,	H-5, 12
12	1.60	172.2	C-4', 5, 6, 7	H-3, 4, 11
13	3.92 ddd (1.6, 9.7, 15.2)	124.8	C-15	H-7, 15
14	5.16 ddd (7.5, 10.1, 15.2)	136.0	-	H-15, 16
15a	1.78 m	39.1	C-13, 14, 16, 17	H-17, 19, 20, 24
15b	2.22 m	-	-	-
16	2.43 m	32.0	C-18	H-14, 24, 25
17	4.05 d (9.7)	122.4	C-16, 19, 24, 25	H-15, 16, 19, 24
18	-	130.7	-	-
19	2.18 m	38.1	C-17, 18, 20, 25	H-17, 20
20a	2.25 m	30.1	C-18, 21, 22	H-15, 20, 25
20b	2.35 m	-	-	-
21	6.81 ddd (5.5, 9.7, 15.2)	150.1	C-23	H-18, 20
22	4.33 d (15.2)	176.0	C-20, 23	H-4, 8
23	-	198.0	-	-
24	0.85 d (6.6)	213.3	C-15, 16, 17	H-15, 16, 17
25	1.30 s	162.3	C-17, 18, 19	H-14, 16, 19, 20,
26	-	-	-	24
27	9.51 s	-	-	-
28	6.98 s	123.2	C-10, 3', 3x, 7x	H-3, 4, 10
29	-	110.7	-	-
30	-	127.0	-	-
31	-	118.3	C-7', 9', 3x, 7x	H-3, 10, 5'
32	7.44 d (7.8)	119.0	C-7', 3x	H-4', 9'
33	6.97 (1.4)	121.6	C-6', 7', 3x, 7x	H-6', 7'
34	7.97 (17.5)	111.3	C-5', 6', 3x	H-6'
35	7.29 d (8.2)	136.4	-	-
36	-	-	-	-
37	-	-	-	-
38	-	-	-	-
39	-	-	-	-
40	-	-	-	-
41	-	-	-	-
42	-	-	-	-
43	-	-	-	-
44	-	-	-	-
45	-	-	-	-
46	-	-	-	-
47	-	-	-	-
48	-	-	-	-
49	-	-	-	-
50	-	-	-	-
51	-	-	-	-
52	-	-	-	-
53	-	-	-	-
54	-	-	-	-
55	-	-	-	-
56	-	-	-	-
57	-	-	-	-
58	-	-	-	-
59	-	-	-	-
60	-	-	-	-
61	-	-	-	-
62	-	-	-	-
63	-	-	-	-
64	-	-	-	-
65	-	-	-	-

^aJ values (in Hz) in parentheses.

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*Multiplicities were determined by analyses of the DEPT spectrum.

Table 2. Cytotoxicity of the isolated compounds 1-9

Compound	Cytotoxicity (IC ₅₀ , μM)		
	BC1 ^a	(KKU-100) ^b	KKU-OC17 ^c
1	5.22	86.95	53.21
2	21.29	85.74	51.95
3	2.54	inactive ^d	inactive ^d
4	3.03	69.89	57.77
5	6.27	inactive ^d	29.66
6	7.20	3.41	12.12
7	20.08	42.42	88.64
8	10.46	29.85	inactive ^d
9	5.51	7.41	85.36
ellipticine	1.06	-	-
5-fluorouracil (5-FU)	348	2.31	-

^aHuman breast cancer cells.
^bPoorly differentiated adenocarcinoma.
^cWell differentiated adenocarcinoma.
^dInactive at > 100.00 μM.

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PUBLICATION

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