UNIVERSITY OF NAIROBI
CHEMISTRY DEPARTMENT

ANTIPLASMODIAL ANTHRAQUINONES AND BENZALDEHYDE DERIVATIVES FROM THE ROOTS OF *KNIPHOFIA THOMSONII*

BY

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OF THE DEGREE OF MASTER OF SCIENCE OF THE
UNIVERSITY OF NAIROBI

2009

This thesis is my original work and has never been presented for a degree in any university.

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DEDICATION

THIS THESIS IS DEDICATED TO MY HUSBAND PETER AND SON LEON.

"Time is too slow for those who wait

Too swift for those who fear

Too long for those who grieve

Too short for those who rejoice

But for those who love

Time is Eternity"

Emma

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I extend my heartfelt appreciation to my entire extended family and more so to my dearest husband, Peter and son, Leon for being there for me whenever I needed them for indeed "true happiness is not attained through self gratification but through fidelity to a worthy purpose". Above all I thank God for my life.

LIST OF ABBREVIATIONS AND SYMBOLS

brs	Broad singlet
bdd	Broad double of a doublet
COSY	Correlated spectroscopy
d	Doublet
dd	Double of a doublet
1D	One dimensional analysis
2D	Two dimensional analysis
ED_{50}	Concentration of 50% Effectiveness
EIMS	Electron ionization mass spectroscopy
F	Fruit
Fl	Flowers
HMQC	Heteronuclear multiple quantum coherence ($^1J_{CH}$)
HMBC	Heteronuclear multiple bond correlation ($^2J_{CH}$, $^3J_{CH}$, $^4J_{CH}$)
Hz	Hertz
IC_{50}	Concentration of 50% inhibition
J	Coupling constant
L	Leaf
MS	Mass spectroscopy
m/z	Mass to Charge ratio
MHz	Mega hertz
m	Multiplet
$[M]^+$	Molecular ion
mp	Melting point
nm	Nanometer
NOESY	Nuclear overhauser and exchange spectroscopy
NMR	Nuclear magnetic resonance
PTLC	Preparative thin layer chromatography
Rh	Rhizomes
R	Roots
s	Singlet
S	Stem
t	Triplet
TLC	Thin layer chromatography
UV	Ultra violet
λ_{max}	Maximum wavelength of absorption

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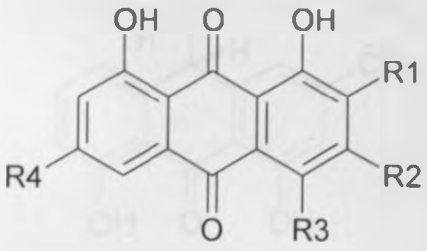
ABSTRACT

The roots of *Knifophia thomsonii* (Asphodelaceae) were exhaustively extracted with dichloromethane/methanol (1:1) by cold percolation at room temperature. The extract showed significant antiplasmodial activity against the chloroquine-resistant (W2) strain of *Plasmodium falciparum* with IC_{50} values of 6.36 µg/ml. The extract was subjected to chromatographic separation which led to the isolation of thirteen secondary metabolites.

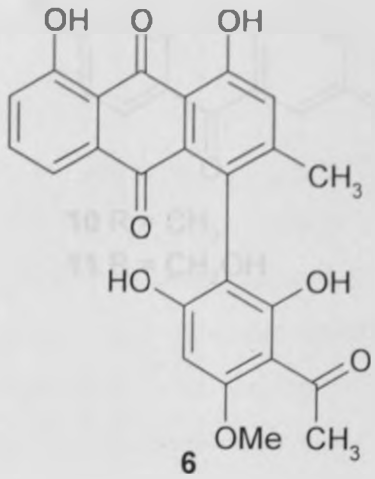
By the use of 1D (^{1}H and ^{13}C) and 2D (COSY, HMBC and HMQC) NMR, MS, UV spectroscopy and direct TLC comparison with authentic samples in some cases, these compounds were identified as the monomeric anthraquinones: chrysophanol (**1**), islandicin (**2**), physcion (**3**), aloe-emodin acetate (**4**) and aloe-emodin (**5**); the phenylanthraquinone: knipholone (**6**); the benzaldehyde derivatives: flavoglaucin (**7**) and 3''',4'''-dehydroflavoglaucin (**8**) and the dimeric anthraquinones: 10,10'-bichrysophanolanthrone (**9**), 10-hydroxy-10-(chrysophanol-7'-yl)-chrysophanolanthrone (**10**), 10-hydroxy-10-(chrysophanol-7'-yl)-aloe-emodinanthrone (**11**), 10-hydroxy-10-(islandicin-7'-yl)-chrysophanolanthrone (**12**) and 10-hydroxy-10-(islandicin-7'-yl)-aloe-emodinanthrone (**13**). The dimeric anthraquinone **13** is a new compound while flavoglaucin (**7**) and 3''',4'''-dehydroflavoglucin (**8**) are reported here for the first time in higher plants. The C-6 oxygenated anthraquinone physcion (**3**) is reported here for the first time in the family Asphodelaceae; and this is also the first report for the occurrence of compound **9** (10,10'-bichrysophanolanthrone) in the genus *Kniphofia*.

The compounds isolated in this study were tested *in vitro* for anti-plasmodial activities against the chloroquine-resistant (W2) strains of *Plasmodium falciparum*. The monomeric anthraquinones were inactive; while the phenylanthraquinone **6** [IC_{50} 2.50 µg/ml (W2)], the benzaldehyde derivatives **7** [IC_{50} 2.06 µg/ml (W2)] and **8** [IC_{50} 1.93 µg/ml (W2)] and the dimeric anthraquinones **9** [IC_{50} 2.23 µg/ml (W2)] and **12** [IC_{50} 3.42 µg/ml (W2)] showed good activities and appear to be partly responsible for the antiplasmodial activity of the crude extract. This investigation has showed the potential

of dimeric anthraquinones and the benzaldehyde derivatives as lead structures for development of antimalarial drugs.



1 $R^1 = R^3 = R^4 = H$, $R^2 = CH_3$
2 $R^1 = R^4 = H$, $R^2 = CH_3$, $R^3 = OH$
3 $R^1 = R^3 = H$, $R^2 = CH_3$, $R^4 = OCH_3$
4 $R^1 = R^3 = R^4 = H$, $R^2 = CH_2OH$
5 $R^1 = R^3 = R^4 = H$, $R^2 = CH_2OAc$



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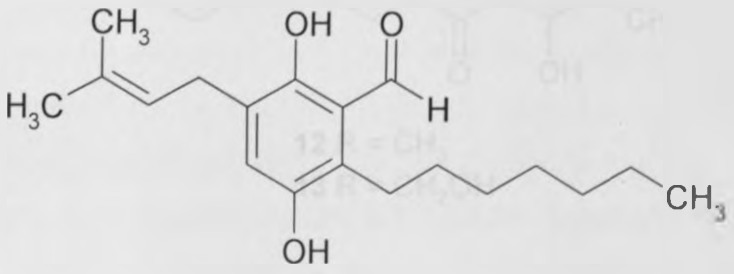

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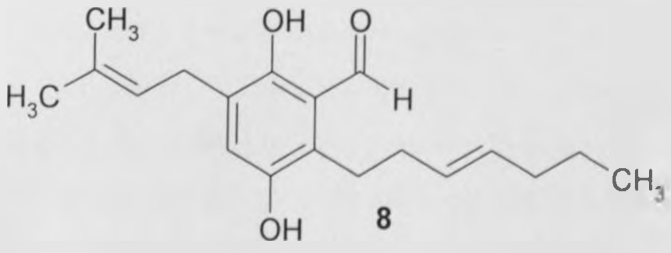

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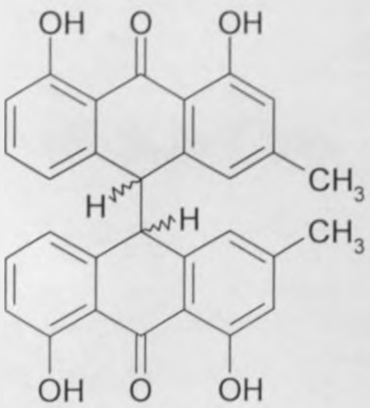

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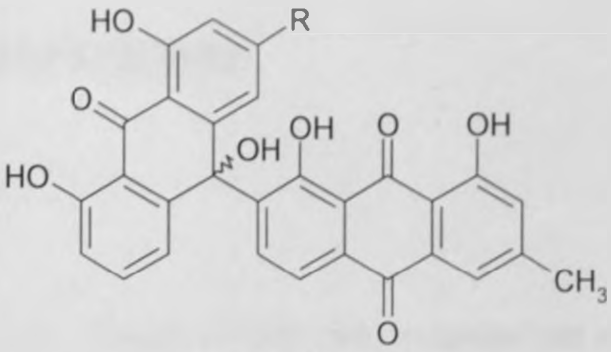

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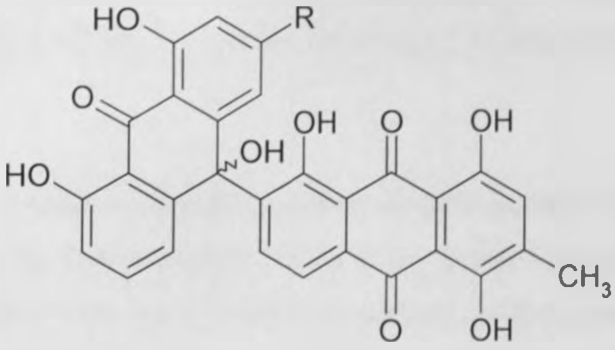


12 R = CH_3
13 R = CH_2OH

CHAPTER ONE

1.0 INTRODUCTION

1.1 General

Since prehistoric time man has gradually, through trial and error, recognized and used plants for the treatment of malaria and other ailments. Healers, elders, parents, or priests passed on orally the knowledge of efficacious traditional medicaments to some members of their family, community, and the next generation. The potential of traditional medicines in improving the health conditions of communities in developing countries by providing the needed medicaments at affordable costs is widely acknowledged (Akerele, 1984).

Currently, in malaria endemic tropical countries, modern medicine is not available, and when it is available, it is not affordable to most of the people living in rural areas. These people resort therefore to the use of traditional medicine as their centerpiece of primary healthcare. This has been so because traditional medicine is commonly available, culturally and socio-economically acceptable, and affordable even in remote rural areas of such populations and communities. The World Health Organization (WHO) estimated that about 80% of the world's population relies on traditional medicine for their primary healthcare needs (Farnsworth *et al.*, 1985).

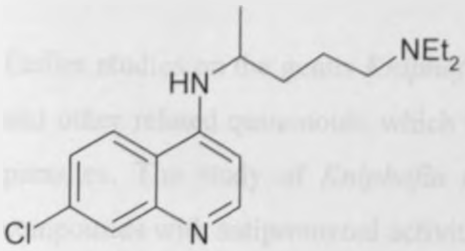
Over one third of the world's population lacks regular access to affordable drugs, such that for these people, modern medicine is unlikely to be a realistic mainstay of their primary healthcare needs. In developing countries, in Africa as well as in South America and Asia, traditional healers are still very often the only medical health care practitioners available to the majority of people living in remote areas (Akubue and Mittal, 1982).

It is estimated that there are 300-500 million cases of malaria annually with 1.75 to 2.5 million deaths (WHO, 2002). Malaria is a leading cause of death in tropical and

subtropical regions and is also a serious public health problem in certain regions of Southeast Asia and South America (Mishra *et al.*, 1999). Several reasons justify the search for new and effective therapeutics for the long-term treatment of malaria. The emergence and spread of insecticide resistant mosquito vectors, and drug resistant parasites are the major reasons. For instance in Kenya, chloroquine (**14**) is no longer effective and the current use of sulfadoxine (**15**) and pyrimethamine (**16**) combination is jeopardized by increased resistance to this combination treatment (Sibley *et al.*, 2001). Although artemisinin-based combination therapies (ACTs) (first line treatment in Kenya currently) have been developed to enhance clinical efficacy and to delay the development of resistance to parasites, these drugs are not yet widely available or affordable.

In this regard research on African traditional medicinal plants for their antimalarials constituents is important, first to facilitate the efficient utilization of the easily available botanical resources to the millions in need and second to provide potentially active lead anti-plasmodial compounds with new mechanisms of action. The interest on study of plants as a source of antimalarial drugs revived due to the fact that one of the most effective antimalarial compound-artemisinin (**18**) (at least as effective as quinine (**17**) and is associated with fewer serious adverse effects) is derived from the plant *Artemisia annua* (Asteraceae) (White *et al.*, 1999).

Natural product research represents a major strategy for discovering and developing new drugs. The use of medicinal plants for the treatment of parasitic diseases is well known and documented since ancient times. The use of *Cinchona succiruba* (Rubiaceae), from which quinine (**17**) was discovered as an antimalarial drug, represented a milestone in the history of drugs from nature used for the treatment of parasitic diseases, especially for those caused by *Plasmodium, Leishmania,* and *Trypanosoma* spp (Oliver *et al.*, 2000).



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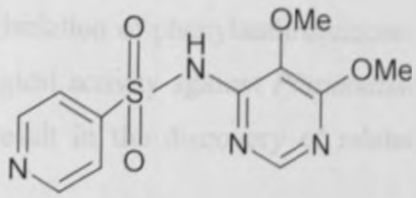

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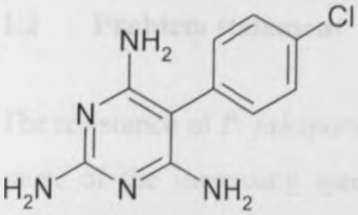

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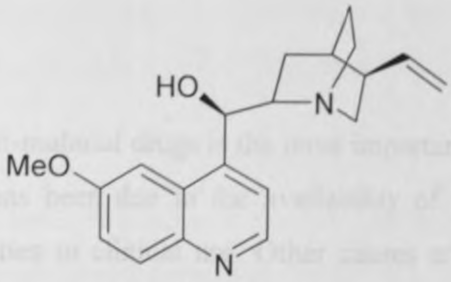

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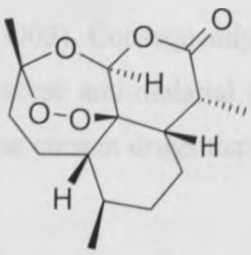

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In the past decades natural products have attracted renewed interest, including from bacteria and fungi, as important sources of biologically active compounds. Recently marine organisms have also been recognized as attractive source of antiparasitic compounds. It is therefore not surprising that one of the frontiers in modern science is the study of the chemistry and biology of natural products (Oliver *et al.*, 2000).

Exploring the untapped natural sources for novel antiplasmodial compounds remains a major challenge and a source of novelty in the era of combinatorial chemistry and genomics. Since plants contain a high variety of constituents it is often claimed that the use of a whole plant rather than one single purified product may be more effective therapeutically (Oliver *et al.*, 2000).

Earlier studies on the genus *Kniphofia* have led to the isolation of phenylanthraquinones and other related quinonoids which have shown biological activity against *Plasmodium* parasites. The study of *Kniphofia thomsonii* could result in the discovery of related compounds with antiprotozoal activities.

1.2 Problem statement

The resistance of *P. falciparum* against first-line anti-malarial drugs is the most important cause of the increasing spread of malaria. This has been due to the availability of a limited number of drugs with few structural varieties in clinical use. Other causes are unaffordable alternative drugs, collapse of vector control programs, resistance of *Anopheles* mosquito against DDT, migration of refugees and changes in the climate and environment (Wiesner *et al.*, 2003). Consequently there is need for the development of efficacious and structurally diverse anti-malarial drugs which may have new modes of action to replace or augment the current drugs that are becoming increasingly ineffective (Ram *et al.*, 2000).

1.3 Justification of the Research

The use of medicinal plants for the treatment of parasitic diseases is well known and documented since ancient times. One of the best examples is the use of *Cinchona succiruba* (Rubiaceae) and related plants as antimalarials. Quinine (**17**) has been identified as the active principle in this plant. This compound and its synthetic derivatives, including chloroquine (**14**), have been used for treatment of malaria for a long time (Oliver *et al.*, 2000). Due to the resistance of *Plasmodium falciparum* to chloroquine and other drugs, there is need for alternative and more effective new drugs to treat malaria and other protozoal diseases. In this regard plants remain the principal source of lead structures.

Phenylanthraquinones are a new class of antiplasmodial (antiprotozoal) compounds (Abegaz *et. al.*, 2002); this remarkable observation was made when it was revealed that knipholone anthrone and related substances possess antiplasmodial activity comparable or only slightly lower than that of chloroquine itself (Bringmann *et. al.*, 1999). Knipholone (**6**) was first reported from the African plant, *Kniphofia foliosa* (Asphodelaceae) (Dagne and Steglich 1984) as the main constituent of the roots. Further work has revealed that anthraquinones including phenylanthraquinones are present in the related genera *Kniphofia* (Dagne and Yenesew, 1994), *Bulbinella* (Van Wyk *et al.*, 1995), and *Bulbine* (Bezabih and Abegaz, 1998).

Many compounds associated with the genus *Kniphofia* have shown several biological activities including antiplasmodial activity. Some of these compounds, especially the phenylanthraquinones and other quinonoids, also have antiprotozoal activities against leishmania parasites (Wube *et al.*, 2006).

In Kenya the only *Kniphofia* species found is *K. thomsonii*. This study was aimed at isolation and characterization of the constituents of this plant and investigation of the isolated compounds for antiplasmodial activities.

1.4 Significance of the Research

This research is expected to contribute in the identification of lead compound(s) with antiplasmodial activities which could lead to cheap and readily available alternative drugs.

1.5 Objectives of the Research

The general objective of this research was to evaluate the antiplasmodial activities of the constituents of the roots of *kniphofia thomsonii*.

The specific objectives of the research were:-

1. To establish the antiprotozoal activity of the roots extract of *Kniphofia thomsonii* against *Plasmodium falciparum*.

2. To isolate and characterize the constituents of the roots extract of *Kniphofia thomsonii*.

3. To determine the antiplasmodial activities of the isolated compounds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria

2.1.1 The Disease

Malaria in humans is caused by a protozoan of the genus *Plasmodium*, which is transmitted through bites by female mosquitoes of the genus *Anopheles*. Four subspecies, namely *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, are known to cause malaria in humans (WHO, 1999).

The most severe malaria fevers and about 90% of malaria deaths are caused by *P. falciparum*, which is the predominant parasite species in Africa (WHO, 1999). It is also in Africa that the most efficient mosquito vector for malaria transmission, *Anopheles gambiae*, predominates (WHO, 1999).

2.1.2 Global Malaria Situation

The global malaria situation is deteriorating faster today than at any time in the past century. The number of new cases of the disease has quadrupled in the past 10 years, such that over 2 billion people, 40% of the world population, living in about 102 countries, are at risk of being infected and half of these live in sub-Saharan Africa. The World Health Organization estimates that between 300 and 500 million new cases occur each year. In addition to causing untold suffering and disability, malaria ranks as one of the world's major killers, costing about 1 million people their lives annually(WHO, 2002).

Children are especially vulnerable, as more children die from malaria than any other single disease. Pregnant women and especially primigravidae (first-time pregnant mothers) are the next highest risk group for malaria in malaria endemic areas. It is stated

CHAPTER TWO

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Children are especially vulnerable, as more children die from malaria than any other single disease. Pregnant women and especially primigravidae (first-time pregnant mothers) are the next highest risk group for malaria in malaria endemic areas. It is stated

that malaria causes about One million deaths of children per year in Africa alone (WHO, 1999). Most malaria infections occur in Africa (Figure 1.1); with countries in tropical Africa estimated to account for 80% of all clinical cases and about 90% of all people who carry the parasite.

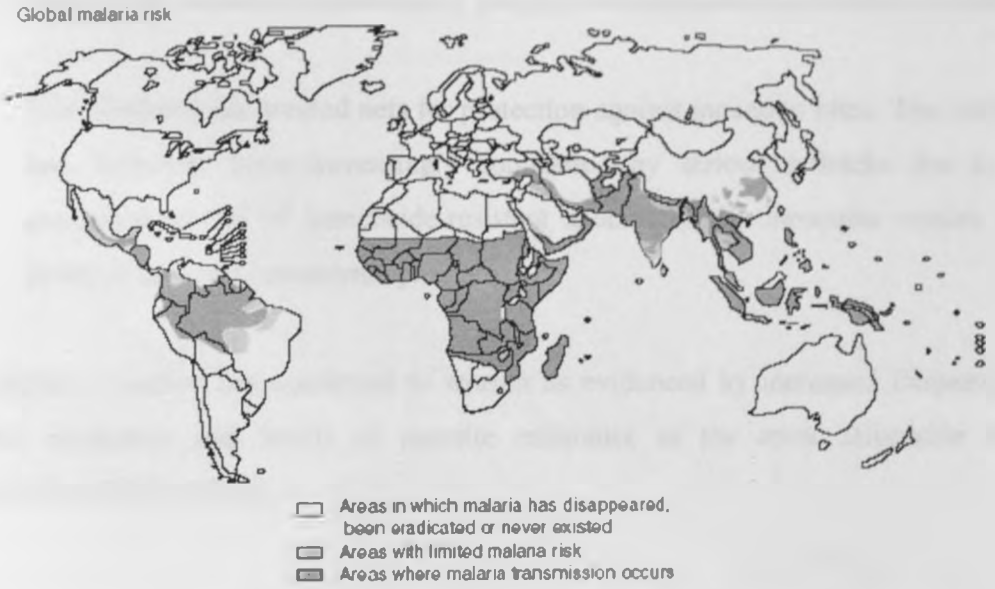


Figure 1.1 Global malaria risks (From 20th WHO Expert Committee Report on Malaria: WHO, 1998)

2.1.3 The Current Malaria Control Strategy

The malaria control strategy aimed at malaria eradication was reoriented in 1978 to focus on the reduction of malaria to a level at which it would no longer constitute a major public health problem (WHO, 1978). This strategy was based on the combined use of vector control measures and effective treatment of malaria patients. This initial success in many tropical countries was interrupted mainly by the development of resistant mosquitoes and chloroquine-resistant *Plasmodium falciparum*, as well as lack of continous political and financial support to the program (Bradley, 1991).

The present malaria control strategy, which was adopted by the Ministerial Conference on Malaria in Amsterdam in 1992 (WHO, 1994), is based on the prevention of death,

reduction of illness, and reduction of social and economic loss due to malaria (WHO, 1994). The practical implementation of the strategy requires two main approaches:

1. Malaria chemotherapy for early and effective treatment of malaria cases, management of severe and complicated cases, and prophylaxis for the most susceptible population (particularly pregnant women and non-immune travelers).

2. Use of insecticide-treated nets for protection against mosquito bites. This strategy has, however, been increasingly confronted by serious setbacks due to the continued spread of insecticide-resistant species of the mosquito vectors, and political and socio-economic problems.

The malaria situation has continued to worsen as evidenced by increased frequency of malaria epidemics and levels of parasite resistance to the most affordable drug, chloroquine (WHO, 1996).

2.1.4 Limitations of the Current Malaria Control Strategy

Currently, chemotherapy and prophylaxis of malaria to those who need it most worldwide is based on nine key drugs: chloroquine, amodiaquine, primaquine, mefloquine, quinine, sulfadoxine/pyrimethamine, pyrimethamine/dapsone, halofantrine, and artemisinin derivatives (WHO, 1998). The availability of chloroquine, amodiaquine, sulfadoxine/pyrimethamine, and quinine in Africa has considerably improved the treatment of malaria. Unfortunately, three decades ago *P. falciparum* was shown to have developed some resistance to most of the cheaper antimalarials. For instance resistance to the 4-aminoquinoline derivative, chloroquine (**14**) was first observed in the 1960s. Resistance to the antifolates pyrimethamine, cycloguanil and mefloquine, has also been reported (Ridley and Hudson, 1998). Since then, the incidence of drug resistant *P. falciparum* has been increasing at a faster rate than that of the efforts for development of new drugs (Figure 1.2).

The most important recent discovery for the therapy of *P. falciparum* malaria has been the identification of artemisinin (**18**) (a sesquiterpene lactone) from *Artemisia annua* (Asteraceae) which has been used to treat over 3 million cases of malaria in South East Asia (Meshnick, 1998). Another new drug, atovaquonehydroxy-naphthoquinone, identified as an antimalarial in the early 1980s, has proved to be highly effective in clinical trials but has to be used in combination with proguanil to prevent the development of resistance (Ridley and Hudson, 1998).

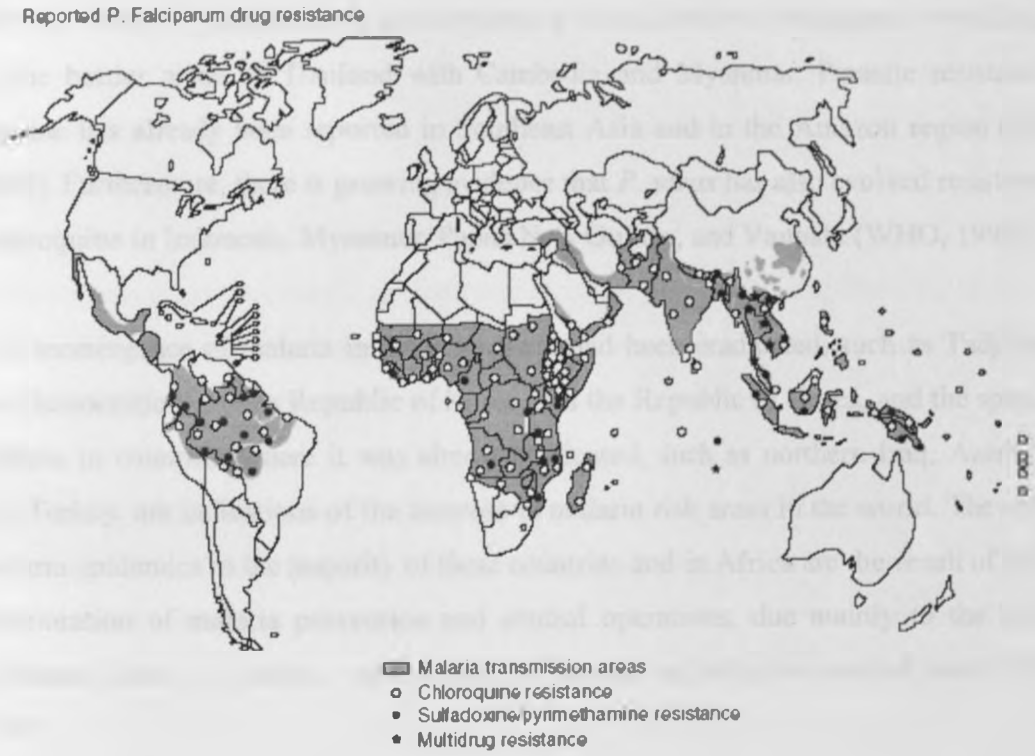


Figure 1.2 Reported *P. falciparum* drug resistance. (From 20th WHO Expert Committee Report on Malaria; WHO, 1998)

Against this background of increasing drug resistance is the unfortunate situation whereby new effective antimalarial drugs coming into the market are completely unaffordable to the majority of the affected populations. Africa south of the Sahara has populations who are dying for lack of malaria treatment, not because there are no effective antimalarial drugs, but because these drugs are beyond their affordability (WHO, 1998).

The spread of chloroquine-resistant *P. falciparum* malaria is a major public health problem in Africa south of the Sahara. Several countries have already abandoned chloroquine as the first-line therapy. Tanzania, Kenya, Malawi, Botswana, and South Africa have switched to sulfadoxine/pyrimethamine, while Cameroon has switched to amodiaquine. Sulfadoxine/pyrimethamine seems to have a short therapeutic life span, as resistant strains of *P. falciparum* are widespread in Southeast Asia and South America (WHO, 1998). Research findings from Kenya and the United Republic of Tanzania indicate a decline in parasite sensitivity to sulfadoxine/pyrimethamine in East Africa (WHO, 1998). *P. falciparum* is also reported to have developed resistance to mefloquine in the border areas of Thailand with Cambodia and Myanmar. Parasite resistance to quinine has already been reported in Southeast Asia and in the Amazon region (WHO, 1998). Furthermore, there is growing evidence that *P. vivax* has also evolved resistance to chloroquine in Indonesia, Myanmar, Papua New Guinea, and Vanuatu (WHO, 1998).

The reemergence of malaria in areas where it had been eradicated, such as Tadjikistan, the Democratic People's Republic of Korea, and the Republic of Korea, and the spread of malaria in countries where it was almost eradicated, such as northern Iraq, Azerbaijan, and Turkey, are indications of the increase in malaria risk areas in the world. The current malaria epidemics in the majority of these countries and in Africa are the result of a rapid deterioration of malaria prevention and control operations, due mainly to the lack of sufficient funds for malaria control and the paucity of effective control tools (WHO, 1998).

The search for a vaccine has been plagued by a number of shortcomings. Many of the shortcomings are related to antigenic variation, antigenic diversity, and immune evasion mechanisms exhibited in various stages of the complex life cycle of malaria parasites. In addition, malaria research and the search for vaccines require large sums of money, and it can be said that malaria research has been greatly under-funded (WHO, 1998).

While efforts are being made to overcome the hurdles for vaccine development, people are dying, and the only available effective means of reducing the number of deaths is the

provision of affordable and effective medicines. Many young people are already dying of HIV/AIDS in Africa for lack of cure and affordable life-prolonging drugs. If, in addition to this, malaria is not controlled using effective drugs, Africa may see the loss of generations of youths and a huge economical setback to the extent that poverty eradication will remain but a dream for ages. The absence of new effective and affordable antimalarials is a formidable limitation to the current malaria control strategy, and there is an urgent need to search for traditional medicines to boost the dwindling number of treatments for malaria (WHO, 1998).

The search for new drugs through the evaluation and validation of traditional medicines offers a good opportunity and a highly credible channel for the discovery and development of better medicines. The advantage of such drugs is that their sources are plants that are often widely available in rural areas of Africa. Furthermore traditional medicine research can provide information and new clues regarding the effectiveness of combination therapy in curing malaria and preventing development of drug resistance (WHO, 2000).

The development of traditional medicines for treatment of malaria, and of African-based pharmaceuticals, would provide the following major benefits to the poorest and worst deprived populations in the world as far as health and economic development is concerned:

- Provision of affordable and effective drugs.
- Prevention of a large number of deaths of children and pregnant women.
- Alleviation of poverty by reducing the burden of malaria and offering the populations alternative commercial crops.
- Creation and strengthening of capacities for drug production.
- A replicable approach to the provision of effective and affordable medicines for other diseases.

The only fear is that if no serious actions are taken to handle the harvesting of such plants, they may soon become extinct because of over harvesting, which is already happening in many cases because of the increased cost of antimalarials (WHO, 2000).

Malaria has a history of the use of plant-based drugs that have saved humankind from many disasters. The natives of Peru discovered the first antimalarial, cinchona bark, and the bark was used in the treatment of intermittent fevers in the 16th century. In 1920 Pelletier and Caventou working in Paris isolated quinine from cinchona bark. Quinine is still one of the best medicines for the treatment of malaria.

The second antimalarial drug is Qinghao (*Artemisia annua)*, used in the treatment of fevers in China. The WHO supported research on *A. annua* and the active principle artemisinin was identified. Artemisinin formulations are used worldwide in the treatment of malaria, particularly for severe and drug-resistant cases (WHO, 1998). Both quinine (**17**) and artemisinin (**18**) are rapid-acting drugs with a short half-life, and therefore resistance against these drugs would be slow. So far there is no reported resistance against artemisinin based drugs.

In India, *Azadirachta indica* (neem) is widely used in the treatment of diseases, including malaria (Sharma *et. al.*, 1993 a; 1993 b). In Africa, the use of indigenous plants still plays an important role in malaria treatment an example being *Kniphofia foliosa* which has been used in Ethiopia for the treatment of malaria (Wube *et al.*, 2006). These plants might be an interesting source for the discovery of novel antiplasmodial lead structures (Onegi *et al.*, 2000).

Anthraquinones isolated from the tropical tree *Morinda lucida* (Rubiaceae) were tested for their antiplasmodial activity (Schnur *et al.*, 1983; Obih *et al.*, 1985). The most active compounds have an aldehyde group at C-2, well known as a cytotoxic moiety in other natural products. The activity may also be explained by the cyclic planar structure that makes them potential DNA-intercalators [molecules that can be reversibly occluded between two other molecules (or groups) or DNA base pairs]. From the toxicological

point of view, some of the compounds showed moderate effects in the lymphocyte proliferation test with EC_{50} values over 175µM (Sittie *et al.*, 1999).

The genus *Kniphofia* is known to contain phenyl anthraquinones which have been shown to possess significant activity against *Leishmania*, *Trypanosoma*, and *Plasmodium* (Schnur. *et al.*, 1983; Obih *et al.*, 1985).

2.2 Botanical Information

2.2.1 The Family Asphodelaceae

The genus *Kniphofia* belongs to the family Asphodelaceae which is comprised of 17 genera (10 of which occur in South Africa) and about 750 species. The family Asphodelaceae is divided into two subfamilies; the Asphodeloideae and the Alooideae. Accordingly the genera *Asphodeline*, *Asphodelus*, *Bulbine*, *Bulbinella*, *Eremurus*, *Hemiphlacus*, *Jodrellia*, *Kniphofia*, *Paradisea*, *Simethis*, and *Trachandra* are placed in the subfamily Asphodeloideae while *Aloe*, *Gasteria*, *Haworthia*, *Lomatophylum*, and *Poellnitzia* are placed in the Alooideae (Stern, 2002).

On the other hand some workers consider the above two subfamilies as distinct families i.e. the Asphodelaceae and the Aloaceae. The generation of chemical information on species belonging to these two groups is believed to reveal the relationships among the various taxa and to assist in establishing taxonomic classifications at various levels (Dagne and Yenesew, 1994).

2.2.1.1 The Sub-family Asphodeloideae

This sub family Asphodeloideae comprises 11 genera with approximately 261 species. They are quite diverse in form ranging from succulent (water-retaining) through mesomorphic (robust) to xenomorphic (not well developed). They feature much less

prominently in collections, but some, such as the "caudiciform"-*Bulbine* and a number of *Kniphofia* genera are popular and quite hardy garden plants (Van Wyk *et al.*, 1995).

2.2.1.1.1 The Genus *Kniphofia*

About 70 species of *Kniphofia* occur in Africa and 47 of these are found in the eastern areas of South Africa. The genus *Kniphofia* is very closely related to the genus *Aloe*. As a result, the first *Kniphofia* to be described, namely *K. uvaria*, was mistakenly thought to be an *Aloe* and was thus initially named *Aloe uvaria* (Stern, 2002).

Most species of *Kniphofia* are evergreen while a few are deciduous and sprout again in the early summer. They bear dense, erect spikes (elongated inflorescence with stalkless flowers) above the level of the leaves in either winter or summer depending on the species. The small, tubular flowers are produced in shades of red, orange, yellow, and cream (Stern, 2002). In Kenya the genus is represented by one species *Kniphofia thomsonii* ("red-hot poker") seen on Mt Kenya, where it was fairly common, and is a native species (Chase *et al.*, 1995).

2.3 *Kniphofia thomsonii*

Tall (1-1.5m) stems with elegant pendulous, well spaced flowers in soft orange terribly beautiful and more intolerant of soggy winter ground (Figure 1.3). This plant usually prefers damp soil, but can also grow in fairly dry conditions (Stern, 2002).

Figure 1.3: Picture of *K. thomsonii*

2.4 Phytochemistry of the Genus *Kniphofia*

The most common class of compounds found in the genus *Kniphofia* are the anthraquinones. Previous phytochemical investigation of this genus resulted in the isolation of monomeric and dimeric anthraquinone.

2.4.1 Monomeric Anthracene Derivatives

The monomeric anthraquinones included chrysophanol (**1**), islandicin (**2**), aloe-emodinacetate (**4**), aloe-emodin (**5**), chrysophanic acid (**23**), chrysophanic acid dianthrone (**24**), emodin (**25**), aloesaponol III (**26**), and aloesaponol III 8-methyl ether (**27**) (Berhanu *et al.* 1986).

2.4.2 Dimeric Anthraquinones

The Dimeric anthraquinones isolated from various *Kniphofia* species include chrysalodin (**11**) from *Kniphofia foliosa* leaves (Dagne *et al.*, 1987), chryslandicin (**12**) from *Kniphofia foliosa* roots (Yenesew *et al.*, 1988) and asphodeline (**19**) from the roots of *K. tysonii* (Van Wyk *et al.*, 1995).

2.4.3 Phenyl Anthraquinone and Phenylanthrones

The unique phenylanthraquinone knipholone **(6)** which was first isolated from *Kniphofia foliosa*, represented the first example in which an anthraquinone is attached to acetylphloroglucinol methyl ether (**30**) unit hence the name phenylanthraquinones. Comparative studies on the roots of some 14 *Kniphofia* species showed knipholone (**6**) to be the major pigment in these taxa. It was therefore suggested that compound **6** may be a taxonomic marker for the genus *Kniphofia* (Yenesew *et al.*, 1988). Studies on other *Kniphofia* species have also resulted in the isolation of a number of phenylanthraquinones and phenylanthrones including isoknipholone anthrone (**21**), isoknipholone (**22**), foliosone (**28**), and isofoliosone (**29**) (Yenesew *et al.*, 1994).

The new phenylanthrone isolated from the stem of *K. foliosa* named knipholone anthrone **(20)**, is reported to be the immediate precursor of knipholone **(6)** (Dagne and Yenesew,

1993). The presence of acetylphloroglucinol methyl ether (**30**) has also been detected in the same plant, a result consistent with the suggestion that knipholone-type compounds arise from oxidative coupling of **30** with a precursor of chrysophanol (**1**). In support of this, other novel pigments where acetylphloroglucinol methyl ether (**30**) attached to C-4 or C-10 positions of the chrysophanol (**1**) moiety have been characterized from this plant (Yenesew *et al.*, 1994). The compounds so far reported from the genus *Kniphofia* are summarized in Table 1.

Table 1: Compounds of the genus *Kniphofia*

Compound	Source species (plant part)	References
Quinonoids		
Knipholone (**6**)	*K. acraea* (R)	Yenesew *et al.* (1988)
	K. caulescens (R)	"
	K. flammula (R)	"
	K. foliosa (L)	Berhanu and Dagne (1984)
	" (R)	Dagne and Steglich (1984)
	" (F, L, Fl)	Berhanu *et al.* (1986)
	" (S)	Yenesew *et al.* (1994)
	" (R)	Yenesew *et al.* (1988)
	K. insignis (Rh)	Berhanu *et al.* (1986)
	K. isoetifolia (Rh)	"
	K. linearifolia (R)	Yenesew *et al.* (1988)
	K. pumila (Rh, Fl)	Berhanu *et al.* (1986)
	K. reynolds (R)	Yenesew *et al.* (1988)
	K. rooperi (R)	"
	K. tysonii (R)	"
	K. schimperi (Rh)	Berhanu *et al.* (1986)
Knipholone anthrone (**20**)	*K. foliosa* (S)	Dagne and Yenesew (1993)
	" (S)	Yenesew *et al.* (1994)
Isoknipholone anthrone (**21**)	" (S)	Yenesew *et al.* (1994)
Isoknipholone (**22**)	*K. foliosa* (S)	Yenesew *et al.* (1994)
Folioosone (**28**)	*K. foliosa* (S)	Yenesew *et al.* (1994)
Isofolioosone (**29**)	*K. foliosa* (S)	Yenesew *et al.* (1994)

KEY: L = Leaf, S = Stem, R = Root, Rh = Rhizomes, Fl = Flowers, F = Fruit

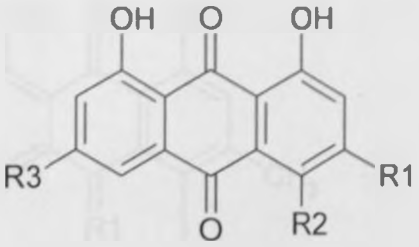
Table 1 Continued.

Compound	Source species (plant part)	References
Asphodeline (**19**)	*K. albescens* (R)	Van Wyk *et al.* (1995)
	K. brachystachya (R)	"
	K. gracilis (R)	"
	K. brevifolia (R)	"
	K. tysonii (R)	"
Chryslandicin (**12**)	*K. caulescens* (R)	Yenesew *et al.* (1988)
	K. foliosa (R)	"
	K. linearifolia (R)	"
Chrysophanic acid (**23**)	*K .caulescens* (R)	Yenesew *et al.* (1988)
	K. foliosa (Rh, F, L, Fl)	Berhanu *et al.* (1986)
	" (R)	Yenesew *et al.* (1988)
	" (L)	Berhanu and Dagne (1984)
	K. insignis (Rh)	Berhanu *et al.* (1986)
	K. isoetifolia (Rh, L, Fl)	"
	K. linearifolia (R)	Yenesew *et al.* (1988)
	K. pumila (Rh)	Berhanu *et al.* (1986)
	K. reynolds (R)	Yenesew *et al.* (1988)
	K. schimperi (Rh)	Berhanu *et al.* (1986)
Chrysophanic acid dianthrone (**24**)	*K. foliosa* (Rh)	Berhanu *et al.* (1985)
	K. insignis (Rh)	"
	K. isoetifolia (Rh)	"
	K. pumila (Rh)	"
	K. schimperi (Rh)	"
Chrysalodin (**11**)	*K. foliosa* (L)	Dagne *et al.* (1987)
Aloe emodin (**5**)	*K. foliosa* (F, L, Fl)	Berhanu *et al.* (1986)
	K. insignis (F)	"
	K. isoetifolia (Fl)	"
	K. schimperi (Fl)	"

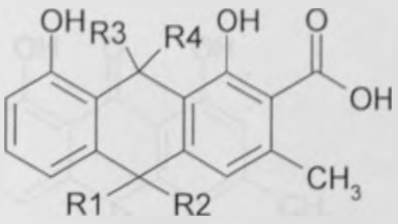
Table 1 Continued.

Compound	Source species (plant part)	References
Aloe emodin acetate (**4**)	*K. foliosa* (L) " (F, L, Fl) *K. isoetifolia* (Fl)	Berhanu and Dagne (1984) Berhanu *et al* (1986) "
Emodin (**25**)	*K. foliosa* (Rh)	Berhanu *et al.* (1986)
Aloesaponol III (**26**)	*K. foliosa* (S)	Yenesew *et al.* (1994)
Aloesaponol III 8-methyl ether (**27**)	*K. foliosa* (S)	Yenesew *et al.* (1994)
Islandicin (**2**)	*K. foliosa* (F, L, Fl) " (R) *K. insignis* (Rh) *K. isoetifolia* (Rh) *K. linearifolia* (R) *K. pumila* (Rh) *K. reynolds* (R) *K. schimperi* (Rh)	Berhanu *et al.*, (1986) Yenesew *et al.*, (1988) Berhanu *et al.* (1986) " Yenesew *et al.*, (1988) Berhanu *et al.* (1986) Yenesew *et al.* (1988) Yenesew *et al.* (1988)
Benzenoid		
Acetophenone,4-6-Dihydroxy-2-methoxy (**30**)	*K. foliosa* (S)	Yenesew *et al.* (1994)
Alkane to C4		
Citric acid (**31**)	*K. butchellii* (L) *K. macoanii* (L)	Van Rheede (1964) "
Malic acid (**32**)	*K. butchellii* (L) *K. macoanii* (L)	Van Rheede (1964) "
Alicyclic		
Quinic acid (**33**)	*K. uvaria* (L)	Yoshida *et al.* (1975)
Shikimic acid (**34**)	*K. uvaria* (L)	Yoshida *et al.* (1975)

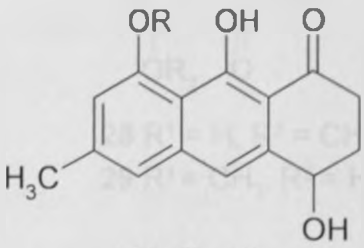
KEY: L = Leaf, S = Stem, R = Root, Rh = Rhizomes, Fl = Flowers, F = Fruit



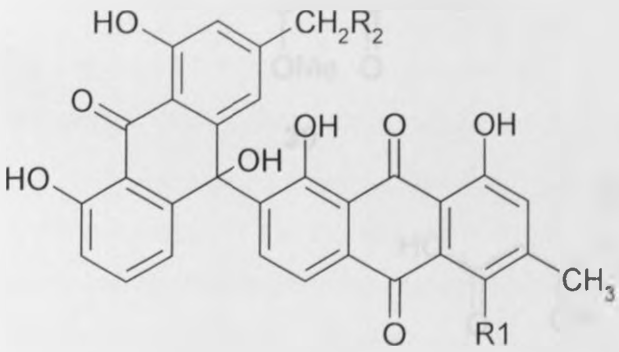
2 $R^1 = CH_3$, $R^2 = OH$, $R^3 = H$
4 $R^1 = CH_2OAc$, $R^2 = R^3 = H$
5 $R^1 = CH_2OH$, $R^2 = R^3 = H$
25 $R^1 = CH_3$, $R^2 = H$, $R^3 = OH$



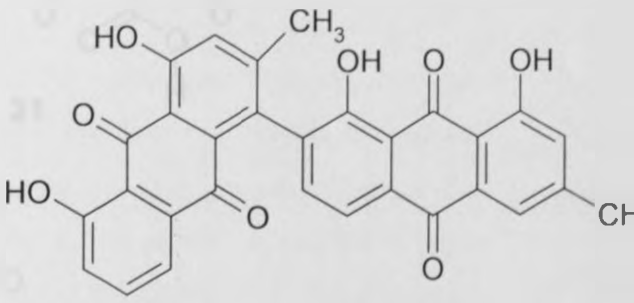
23 $R^1 = R^2 = R^3 = R^4 = O$
24 $R^1 = R^2 = R^3 = R^4 = H$



26 R = H
27 $R = CH_3$



11 $R^1 = H$, $R^2 = OH$
12 $R^1 = OH$, $R^2 = H$



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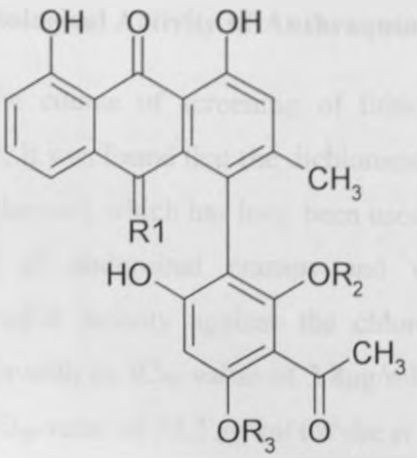
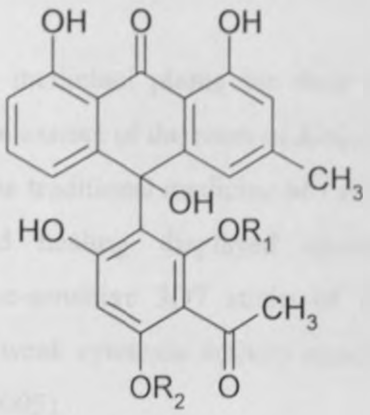
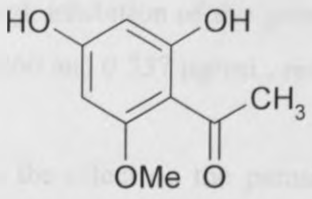


20 $R^1 = 2H$, $R^2 = H$, $R^3 = CH_3$
21 $R^1 = 2H$, $R^2 = CH_3$, $R^3 = H$
22 $R^1 = O$, $R^2 = CH_3$, $R^3 = H$



28 $R^1 = H$, $R^2 = CH_3$
29 $R^1 = CH_3$, $R^2 = H$



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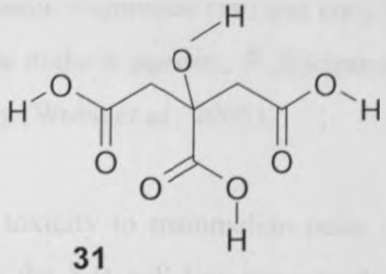

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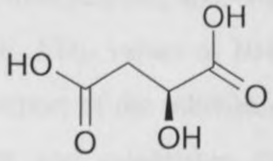

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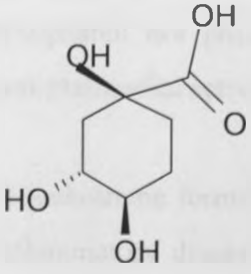

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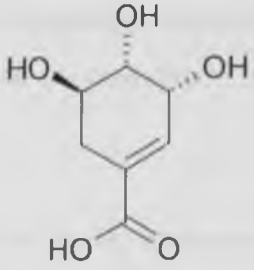


34

2.5 Biological Activity of Anthraquinones

During the course of screening of Ethiopian medicinal plants for their antimalarial properties, it was found that the dichloromethane extract of the roots of *Kniphofia foliosa* (Asphodelaceae), which has long been used in the traditional medicine of Ethiopia for the treatment of abdominal cramps and wound healing, displayed strong *in vitro* antiplasmodial activity against the chloroquine-sensitive 3D7 strain of *Plasmodium falciparum* with an IC_{50} value of 3.8μg/ml and weak cytotoxic activity against KB cells with an ED_{50} value of 35.2 μg/ml (Wube *et al.*, 2005).

The compounds isolated from the roots of *Kniphofia foliosa* (Asphodelaceae) were evaluated for their *in vitro* antimalarial activity against the chloroquine-sensitive 3D7 strain of *Plasmodium falciparum* (Wube *et al.*, 2005). Among the compounds tested, 10-(chrysophanol-7'-yl)-10-(xi)-hydroxychrysopanol-9-anthrone (**10**) and chryslandicin (**12**), showed a high inhibition of the growth of the malaria parasite, *P. falciparum* with ED_{50} values of 0.260 and 0.537 μg/ml , respectively (Wube *et al.*, 2005).

To compare the effect on the parasite with toxicity to mammalian cells, the cytotoxic activities of the isolated compounds against the KB cell line were evaluated and 10-(chrysophanol-7'-yl)-10-(xi)-hydroxychrysopanol-9-anthrone (**10**) and chryslandicin (**12**) displayed very low toxicity with ED_{50} values of 104 and 90 μg/mL, respectively (Wube *et al.*, 2005). This is the first report of the inhibition of the growth of *P. falciparum* by anthraquinone-anthrone dimers and establishes them as a new class of potential antimalarial compounds with very little host cell toxicity (Wube *et al.*, 2005).

Phenylanthraquinones such as knipholone (**6**) have antiplasmodial activities; whereas neither chrysophanol nor phloroacetophenone, which are its monomers, possess any significant antiplasmodial activity (Majinda *et al.*, 2001).

Inhibition of leukotriene formation is one of the approaches for the treatment of asthma and other inflammatory diseases. Knipholone (**6**), isolated from the roots of *Kniphofia foliosa*, (Asphodelaceae), has been investigated for inhibition of leukotriene biosynthesis

in an *ex vivo* bioassay using activated human neutrophile granulocytes. It was found to be a selective inhibitor of leukotriene metabolism in human blood assay where it showed a high dose dependent activity with an IC_{50} value of 4.2 µM (3.6-4.9, 95% FL) (Wube *et al.*, 2006) being twice as active as the commercial 5-LOX (5-Lipoxygenase) inhibitor zileuton (IC_{50} 10.4 µM, 9.0-11.7, 95% FL) (Wube *et al.*, 2006).

Knipholone (**6**) showed weak inhibition of 12(S)-HETE production using human platelets at 10 µg/ml concentration which resulted in 28.6% inhibition. Baicalein was used as a positive control and exhibited 52% inhibition at 5 µg/ml. However, at concentrations of 50 µg/ml, it produced no inhibition of other enzymes related to inflammation, such as COX-1 and -2 compared to the positive controls indomethacin which inhibits COX-1 with an IC_{50} value of 0.9 µM and NS-398 which inhibits COX-2 with an IC_{50} value of 2.6 µM. Therefore, Knipholone showed higher affinity for the 5-LOX (enzyme involved in leukotrienes biosynthesis) pathway than for cycloxygenases and 12-LOX (Wube *et al.*, 2006).

In an attempt to explain the mechanism of inhibition, the antioxidant activity of knipholone using various *in vitro* assay systems including free radical scavenging (stable DPPH), non-enzymatic lipid peroxidation (inhibitory activity on bovine brain liposomes), and metal chelation (Cu^{2+}, Fe^{2+} and Fe^{3+}) was examined. Knipholone (**6**) was found to be a weak dose-independent free radical scavenger with an IC_{50} value of 355 µM compared to positive control quercetin (IC_{50}=3.2 µM). It was found to be a weak inhibitor of phospholipids liposomes peroxidation with an IC_{50} value of 311 µM compared to the positive control quercetin (IC_{50}=1.4 µM). However, with the addition of the metal ions, neither the absorbance nor the intensity of the UV-bands changed even with a concentration of 100 µM of metal ions. Thus Knipholone (**6**) is not a metal chelator. Cytotoxicity results also provided evidence that compound **6** exhibits weak toxicity for a mammalian host cell with ED_{50} value of 0.58 µM (Wube *et al.*, 2006). Therefore, the *ex vivo* leukotriene metabolism inhibitory capacity of knipholone (**6**) is powerful and could be a potential candidate for a new anti-asthma drug (Wube *et al.*, 2006).

CHAPTER THREE

3.0 METHODOLOGY

3.1 General

3.1.1 Instrumentation

The ^{13}C NMR (125 or 50 MHz) and ^{1}H NMR (300 or 200 MHz) were run on Bruker or Varian-Mercury spectrometers using TMS as the internal standard. Homonuclear correlation spectroscopy (COSY), Nuclear overhauser Enhancement spectroscopy (NOESY), Heteronuclear correlation spectroscopy (HETCOR) including HMBC ($^{2}J_{CH}$, $^{3}J_{CH}$) and heteronuclear multiple quantum coherence ($^{1}J_{CH}$) HMQC were acquired using standard Bruker software. UV/VIS spectra were recorded using a Pye-Unicam SPS-150 Spectrophotometer. The roots were ground using willymill.

3.1.2 Collection of Plant Material

The roots of *Kniphofia thomsonii* was collected with the help of botanists from the Department of Botany, University of Nairobi. The area of collection was Mt. Kenya. The material was authenticated at the Department of Botany, University of Nairobi.

3.1.3 Chromatographic conditions

Compounds were isolated mainly from the bioactive extracts using various chromatographic techniques including column chromatography (oxalic acid impregnated) and Sephadex LH-20. Preparative thin layer chromatography (PTLC) using silica gel as the adsorbent and crystallization of solid compounds were used in the final purification. Analytical TLC was done on Merck pre-coated silica gel 60 F_{254} plates, with UV (254 nm) and iodine vapour as detectors.

The spectroscopic methods used to determine the molecular structures of pure compounds isolated were ultra violet spectroscopy (UV), NMR spectroscopy and mass spectroscopy (MS) techniques.

3.2 Extraction and Isolation of compounds

Air dried and ground roots of *Kniphofia thomsonii* (1.0 Kg) were extracted with dichloromethane/methanol (1:1) by cold percolation. The extract was evaporated under reduced pressure to yield 105 g (10.5%) crude extract. About 100 g of the extract was subjected to column chromatography on oxalic acid impregnated silica gel (400 g) eluting at gradient with hexane, dichloromethane and methanol to afford 11 combined fractions labeled 1A-1K.

Subsequent column chromatography of fraction 1B (2.027 g) on oxalic acid impregnated silica (eluting with 3%, 5%, 7% 10%, 15% and 30% dichloromethane in hexane) and crystallization (from n-hexane/dichloromethane), gave chrysophanol (**1**) (30 mg), flavoglaucin (**7**) (30.3 mg) and 10,10`-bichrysophanol anthrone (**9**) (29 mg) respectively. Fraction eluting with 3% dichloromethane in hexane was subjected to Sephadex LH-20, CH_2Cl_2/CH_3OH (1:1) to yield 1,4,8-trihydroxy-3-methylanthraquinone, trivial name islandicin (**2**) (5 mg).

Combined fractions eluting with 3%, 5%, 7% and 10% dichloromethane in hexane, were subjected to Sephadex LH-20 and further purified using PTLC (preparatory thin layer chromatography). This gave 1,8-dihydroxy-3-methyl-6-methoxyanthraquinone, trivial name physcion (**3**) (5 mg), 3```,4```-dehydroflavoglaucin (**8**) (6 mg) was achieved after subjecting the 15% dichloromethane in hexane extract from fraction 1B to Sephadex LH-20 and further purification using PTLC.

Fractions 1C-1H of the first column was combined (8Q) to yield a weight of 11 g. This was then subjected to column chromatography (200 g-oxalic acid impregnated silica gel) using n-hexane containing increasing amounts of dichloromethane and ethyl acetate. This

gave a total of 17 fractions labeled 10A-10Q. Crystallization (from n-hexane/dichloromethane) of fraction 10H eluting with 100% dichloromethane gave rise to 10-hydroxy-10-(islandicin-7'-yl)-chrysophanol anthrone with the trivial name chryslandicin **12** (21 mg). The mother liquor of 10H together with fractions 10I and 10J were combined and labeled 14A (1.06g). Subsequent column chromatography on oxalic acid impregnated silica gel (60g) gave 15A-15V. Fraction 15G eluting with 1:1 dichloromethane/hexane was subjected to Sephadex LH-20, CH_2Cl_2/CH_3OH (1:1) and PTLC giving rise to aloe-emodin acetate **5** (5.2 mg).

Combined fractions of 10K-10P and 15M-15N (5 g) labeled 20G was subjected to column chromatography (oxalic acid impregnated silica gel-70g) using n-hexane containing increasing amounts of dichloromethane and ethyl acetate realized 19 fractions labeled 21A-21S. Fractions 21G-21I were combined and labeled 23A. Further Sephadex LH-20 [CH_2Cl_2/CH_3OH (1:1)] and PTLC on fraction 23A gave 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methylanthraquinone, trivial name knipholone **6** (5 mg) and 10-hydroxy-10-(chrysophanol-7'-yl) -chrysophanol anthrone **10** (11 mg). Fraction 21S eluting with 50% ethyl acetate in hexane was also subjected through several Sephadex LH-20 [CH_2Cl_2/CH_3OH (1:1)] and PTLC and gave rise to aloe-emodin **4** (8 mg), 10-hydroxy-10-(chrysophanol-7'-yl)-aloe-emodin anthrone (Chrysalodin) 11 (10 mg), and 10-hydroxy-10-(islandicin-7'-yl)-aloe-emodin anthrone **13** (7 mg).

3.3 Physical and Spectroscopic Data for the Isolated Compounds

3.3.1 Chrysophanol (1)

Orange crystals, melting point 195-197°C; UV (λ_{max}, MeOH): 270, 288 and 430 nm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 7.08 (1H, *bdd*, J = 7.8, 1.5 Hz, H-2), 7.62 (1H, *bd* J = 1.8 Hz, H-4), 7.80 (1H, *dd*, J = 7.5, 1.2 Hz, H-5), 7.65 (1H, *t*, J = 8.4 Hz, H-6), 7.28 (1H, *dd*, J = 7.8, 1.5 Hz, H-7), 2.45 (3H, *s*, Me-3), 11.98 (OH-1), 12.09 (OH-8).

3.3.2 Islandicin (2)

Glittering red crystals, melting point 217-219°C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 7.15 (1H, *q*, *J* = 0.9 Hz, H-2), 7.89 (1H, *dd*, *J* = 7.5, 1.2 Hz, H-5), 7.68 (1H, *t*, *J* = 7.5Hz, H-6), 7.87 (1H, *dd*, *J* = 7.5, 1.2 Hz, H-7), 2.38 (3H, *d*, Me-3) , 13.48 (1-OH, C-4), 12.28 (*2-OH, C-1 and C-8),

3.3.3 Physcion (3)

Yellow powder: ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 7.09 (1H, *q*, H-2) , 7.64 (1H, *bd*, H-4), 7.38 (1H, *d*, *J* = 2 Hz, H-5), 6.70 (1H, *d*, *J* = 2 Hz, H-7), 2.46 (3H, *s*, Me-3), 3.92 (3H, *s*, OMe-6), 12.33 (OH-1), 12.13 (OH-8).

3.3.4 Aloe-emodin (4)

Yellow amorphous solid: UV (λ_{max}, MeOH) 271 and 429 nm; ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.37 (1H, *J* = 1.5 Hz, H-2), 7.80 (1H, *d*, *J* = 1.0 Hz, H-4), 7.80 (1H, *d*, *J* = 1.0, 8.0 Hz, H-5), 7.83 (1H, *t*, *J* = 8.0 Hz, H-6) 7.37 (1H, *d*, *J* = 1.5, 8.0 Hz, H-7), 4.81 (2H, *s*,CH_2OH-3), 12.05 (OH-1), 12.01 (OH-8); ^{13}C NMR (ACETONE-d_6, 125 MHz): δ_C 164.1 (C-1), 117.9 (C-1a), 125.8 (C-2), 155.6 (C-3), 120.1 (C-4), 138.1 (C-4a), 121.1 (C-5), 137.9 (C-5a), 138.97 (C-6), 122.3 (C-7), 163.7 (C-8), 117.6 (C-8a), 194.5 (C-9), 182.9 (C-10), 64.4 (CH_2OH-3).

3.3.5 Aloe-emodin acetate (5)

Yellow amorphous solid: UV (λ_{max}, MeOH) 268 and 429 nm; ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.27 (*s*, H-2), 7.79 (1H, *s*, H-4), 7.85 (1H, *d*, *J* =8.0 Hz, H-5), 7.70 (1H, *t*, *J* = 8.0 Hz, H-6), 7.32 (1H, *d*, *J* = 8.0 Hz, H-7), 5.19 (2H, *s*, $CH_2OC(O)CH_3$-3), 2.19 (3H, *s*, $CH_3(O)OCH_2$-3), 12.06 (OH-1), 12.08 (OH-8); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 162.8 (C-1), 115.8 (C-1a), 122.4 (C-2), 146.5 (C-3), 118.5 (C-4), 133.5 (C-4a), 120.2 (C-5), 133.9 (C-5a), 137.3 (C-6), 124.8 (C-7), 162.6 (C-8), 115.3 (C-8a), 192.7 (C-9), 181.5 (C-10), 170.0 (C(O)-3), 20.8 ($OC(O)CH_3$-3), 64.7 (-$CH_2OC(O)$-3).

3.3.6 Knipholone (6)

Deep red needles; UV (λ_{max}, MeOH) 224, 246, 254, 271, 289, 369 and 430 nm, ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 12.60 (OH-1), 11.99 (OH-8), 7.55 (1H, *dd*, *J* = 8.0, 1.1 Hz, H-5), δ_H 7.75 (1H, *t*, *J* = 8.0 Hz, H-6), 7.29 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-7), 7.36 (1H, s, H-2), 6.24 (1H, *s*, H-5'), 2.17 (3H, *s*, CH_3-3), 14.20 (OH-2'), 2.63 (3H, *s*, $CH_3C(O)$-3'), 3.98 (3H, *s*, OCH_3-4').

3.3.7 Flavoglaucin (7)

Yellow amorphous solid; EIMS (*m/z* 304, $C_{19}H_{28}O_3$); UV (λ_{max}, MeOH) 274 and 391 nm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 11.93 (OH-1), 6.90 (1H, *s*, H-5), 4.38 (*s*, OH-4), 10.24 (1H, *s*, H-1'), 3.29 (2H, *d*, *J* = 7.5 Hz CH_2-1"), 5.28 (1H, *t*, *J* = 7.5, 17 Hz, CH-2"), 1.76 (3H, *bs*, CH_3-3"), 1.70 (3H, *bs*, CH_3-3"), 2.91 (2H, *t*, CH_2-1'''), 1.58 (2H, *m*, CH_2-2'''), 1.36 (8H, *m*, CH_2-4), 0.90 (3H, *t*, CH_3-7'''); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 155.8 (C-1), 117.3 (C-2), 128.6 (C-3), 144.97 (C-4), 125.7 (C-5), 128.6 (C-6), 195.5 (C-1'), 27.0 (C1"), 121.2 (C-2"), 133.6 (C-3"), 17.8 (CH_3-3"), 25.8 (CH_3-3"), 23.9 (C-1'''), 31.99 (C-2'''), 29.6 (C-3'''), 29.1 (C-4'''), 31.99 (5'''), 22.6 (C-6'''), 14.1 (C-7''').

3.3.8 3''',4'''-Dehydroflavoglaucin (8)

Yellow amorphous solid; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 11.95 (OH-1), 6.90 (1H, *s*, H-5), 4.38 (*s*, OH-4), 10.23 (1H, *s*, H-1'), 3.30 (2H, *d*, *J* = 7.2 Hz CH_2-1"), 5.28 (1H, *t*, *J* = 7.2, 17.4 Hz, CH-2"), 1.76 (3H, *bs*, CH_3-3"), 1.70 (3H, *bs*, CH_3-3"), 2.96 (2H, *t*, CH_2-1'''), 2.28 (2H, *bq*, CH_2-2'''), 5.51 [$(2H)_2$, *t*, CH-3'''; CH-4'''], 1.99 (2H, *bq*, CH_2-5'''), 1.34 (2H, *m*, CH_2-6''') and 0.87 (3H, *t*, CH_3-7''').

3.3.9 10,10'-Bichrysophanol anthrone (9)

Pale-yellow amorphous powder; HRMS [*m/z*, (rel. Int.)]: M^+ 479.1483 (100), $C_{30}H_{23}O_6$; EIMS [*m/z*, (rel. Int.)]: M^+ 479 (5), 478 (15), 241 (17), 240 (85), 239 (100); UV (λ_{max}, MeOH): 271 and 367 nm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 2.32 (3H, s, CH_3-3), 4.49 (1H, *d*, *J* = 1.5 Hz, H-10), 6.65 (1H, *brs*, H-2), 6.15 (1H, *brs*, H-4), 6.73 (1H, *dd*, *J* = 7.5, 1.2 Hz, H-5), δ_H 7.50 (1H, *t*, *J* = 7.5 Hz, H-6) 6.94 (1H, *dd*, *J* = 7.5, 1. 2 Hz, H-7), 2.22 (3H, s, CH_3-3'), 4.45 (1H, *d*, *J* = 1.5Hz, H-10'), 6.65 (1H, *brs*, H-2'), 5.73 (1H, *brs*, H-4'),

6.33 (1H, *d*, *J* = 8.0 Hz, H-5'), 7.30 (1H, *t*, *J* = 8.0 Hz, H-6'), 6.87 (1H, *d*, *J* = 8.0 Hz, H-7'), 11.71 (OH-1), 11.63 (OH-1'), 11.88 (OH-8), 11.79 (OH-8'); ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 161.9 (C-1), 114.0 (C-1a), 117.0 (C-2), 147.5 (C-3), 121.0 (C-4), 141.0 (C-4a), 117.0 (C-5), 142.1 (C-5a), 135.7 (C-6), 116.8 (C-7), 162.1 (C-8), 117.2 (C-8a), 191.6 (C-9), 56.3 (C-10), 22.1 (CH_3-3), 161.9 (C-1'), 114.5 (C-1a'), 116.9 (C-2'), 147.3 (C-3'), 121.2 (C-4'), 140.9 (C-4a'), 119.4 (C-5'), 142.1 (C-5a'), 135.3 (C-6'), 116.9 (C-7'), 162.2 (C-8'), 116.7 (C-8a'), 191.6 (C-9'), 56.3 (C-10'), 21.9 (CH_3-3').

3.3.10 10-Hydroxy-10-(chrysophanol-7'-yl)-chrysophanol anthrone (10)

Yellow-red amorphous powder: HRMS [*m/z*, (rel. Int.)]: ([M+1]$^+$ 509.1253 (100), $C_{30}H_{20}O_8$); EIMS [*m/z*, (rel. Int.)]: M$^+$ 508 (21), 490 (30), 475 (26), 255 (19), 85 (55), 57 (100); UV (λ_{max}, MeOH): 271, 292, 389 and 434 nm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 2.23 (3H, *s*, CH_3-3), 7.59 (1H, *brs* H-2), 7.12 (1H, *brs*, H-4), 6.77 (1H, *bs*, H-5), 7.50 (1H, *t*, *J* = 8.0 Hz, H-6), 6.91 (1H, *d*, *J* = 8.0, 1.2 Hz, H-7), 2.45 (3H, *s*, CH_3-3'), 6.77 (1H, *bs*, H-2'), 6.88 (1H, *bs*, H-4'), 7.98 (1H, *bs*, H-5'), 8.81 (1H, *bs*, H-6'), 12.24 (*s*, OH-1), 12.32 (*s*, OH-1'), 12.26 (*s*, OH-8), 12.34 (*s*, OH-8'), 5.6 (*s*, OH-10); ^{13}C NMR (ACETONE-d_6, 125 MHz): δ_C 164.2 (C-1), 115.4 (C-1a), 122.1 (C-2), 149.2 (C-3), 125.5 (C-4), 146.9 (C-4a), 118.6 (C-5), 146.9 (C-5a), 118.6 (C-6), 118.3 (C-7), 164.2 (C-8), 116.1 (C-8a), 194.9 (C-9), 75.1 (C-10), 22.79 (CH_3-3), 163.9 (C-1'), 114.0 (C-1a'), 122.3 (C-2'), 150.3 (C-3'), 120.9 (C-4'), 132.7 (C-4a'), 120.7 (C-5'), 132.7 (C-5a'), 135.1 (C-6'), 130.3 (C-7'), 163.7 (C-8'), 116.1 (C-8a'), 182.8 (C-9'), 202.0 (C-10'), 23.8 (CH_3-3').

3.3.11 10-Hydroxy-10-(chrysophanol-7'-yl)-aloe-emodin anthrone (11)

Yellow amorphous: HRMS [*m/z*, (rel. Int.)] ([M+1]$^+$ 525.1185 (100), $C_{30}H_{21}O_9$); EIMS [*m/z*, (rel. Int.)]: M$^+$ 525 (5), 524 (16), 506 (15), 476 (37), 254 (21), 239 (19), 190 (45), 149 (67), 57 (79), 43 (100); UV (λ_{max}, MeOH): 271, 292, 385 and 494 nm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 6.96 (1H, *bd*, *J* = 1.0 Hz, H-2), 6.68 (1H, *bd*, *J* = 2.5Hz, H-4), 6.96 (1H, *bd*, *J* = 8.0 Hz, H-5), 7.51 (1H, *t*, *J* = 8.0 Hz, H-6), 6.92 (1H, *bd*, *J* = 8.0 Hz, H-7), 4.56 (2H, bs, CH_2OH-3), 7.58 (1H, *s*, H-2').and 7.11 (1H, *s*, H-4'), 7.98 (1H, *d*, *J* = 7.6 Hz, H-5') and 8.81 (1H, *d*, *J* = 7.6 Hz, H-6'), 2.46 (3H, *bs*, CH_3-3'), 12.29 (*s*, OH-1), 12.30 (*s*, OH-8), 12.32 (*s*, OH-1'), 12.34 (*s*, OH-8'), 5.91 (*s*, OH-10); ^{13}C NMR

(ACETONE-d_6, 125 MHz): δ_C 163.5 (C-1), 116.7 (C-1a), 114.6 (C-2), 153.9 (C-3), 120.3 (C-4), 143.1 (C-4a), 117.2 (C-5), 137.7 (C-6), 114.8 (C-7), 163.4 (C-8), 117.6 (C-8a), 194.4 (C-9), 63.8 (CH_2OH-3), 163.0 (C-1'), 114.1 (C-1a), 124.8 (C-2'), 143.1 (C-3'), 117.9 (C-4'), 134.0 (C-4a'), 120.3 (C-5'), 133.8 (C-6'), 121.5 (C-7') 160.5 (C-8'), 117.5 (C-8a), 182.1 (C-9'), 179.3 (C-10'), 22.1 (CH_3-3').

3.3.12 10-Hydroxy-10-(islandicin-7'-yl)-chrysophanol anthrone (12)

Red amorphous powder: HRMS [*m/z*, (rel. Int.)] ([M+1]$^+$ 525.1211 (100), $C_{30}H_{20}O_9$); EIMS [*m/z*, (rel. Int.)]: M$^+$ 525 (2), 510 (5), 509 (12), 508 (22), 507 (30), 476 (7), 254 (15), 240 (32), 239 (17), 197 (11), 149 (35), 57 (100); UV (λ_{max}, MeOH): 271, 299, 381 and 495 nm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 6.78 (1H, *bs*, H-2), 6.61 (1H, *bs*, H-4), 6.80 (1H, *dd*, *J* = 8.0, 1.2 Hz, H-5), 7.41 (1H, *t*, *J* = 8.0 Hz, H-6), 6.95 (1H, *dd*, *J* = 8.0, 1.2 Hz, H-7), 2.26 (3H, s, CH_3-3), 7.10 (1H, *s*, H-2'), 8.06 (1H, *d*, *J* = 8.0 Hz, 5'), 8 66 (1H, *d*, *J* = 8.0 Hz, H-6'), 2.35 (3H, *s*, CH_3-3'), 12.08 (OH-1, *s*), 12.32 (OH-8, *s*), 12.34 (OH-1', *s*), 13.56 (OH-4', *s*), 12.44 (OH-8', *s*); ^{13}C NMR (ACETONE-d_6, 125 MHz): δ_C 163.5 (C-1), 114.2 (C-1a), 118.5 (C-2), 150.3 (C-3), 122.2 (C-4), 143.3 (C-4a), 121.1 (C-5), 149.0 (C-5a), 138.2 (C-6), 118. 2 (C-7), 163.8 (C-8), 195.0 (C-9), 24.4 (CH_3-3), 158.9 (C-1'), 112.5 (C-1a'), 130.4 (C-2'), 149.5 (C-3'), 158.9 (C-4'), 112.5 (C-4a'), 120.4 (C-5'), 134.6 (C-5a), 134.4 (C-6'), 160.0 (C-8'), 188.2 (C-9'), 188.2 (C-10'), 24.1 (CH_3-3').

3.3.13 10-Hydroxy-10-(islandicin-7'-yl)-aloe-emodin anthrone (13)

Red amorphous solid: HRMS [*m/z*, (rel. Int.)] ([M+1]$^+$ 541.1130, (100) $C_{30}H_{20}O_{10}$); EIMS [*m/z*, (rel. Int.)]: M$^+$ 540 (3), 523 (5), 492 (100), 447 (3), 417 (2), 321 (3), 283 (5), 267 (7), 57 (74), 43 (85); UV (λ_{max}, MeOH): 271, 298, 381and 495 nm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 6.95 (1H, *brs*, H-2), 6.90 (1H, *brs*, *J* = 0.6 Hz, H-4), 6.94 (1H, *bd*, *J* = 8.5 Hz, H-5), 7.51 (1H, *t*, *J* = 8.0 Hz, H-6), 6.92 (1H, *bd*, *J* = 8.5 Hz, H-7), 4.56 (2H, *bs*, CH_2OH-3), 7.21 (1H, *brs*, H-2'), 8.06 (1H, *brs*, H-5'), 8.83 (1H, *brs*, H-6'), 2.28 (3H, *s*, CH3-3'), 12.29 (*s*, OH-1), 12.31 (*s*, OH-8), 12.33 (*s*, OH-1'), 13.51 (*s*, OH-4'), 12.34 (*s*, OH-8'), 6.05 (*s*, OH-10); ^{13}C NMR (ACETONE-d_6, 125 MHz): δ_C 169.3 (C-1), 114.1 (C-1a), 114.6 (C-2), 153.9 (C-3), 120.4 (C-4), 148.1 (C-4a), 117.9 (C-5), 148.1 (C-5a), 137.7 (C-6), 114.7 (C-7), 163.0 (C-8), 202.8 (C-9), 63.8 (CH_2OH-3), 165.9 (C-1'), 111.7 (C-

1a'), 129.9 (C-2'), 144.0 (C-3'), 159.7 (C-4'), 112.6 (C-4a'), 120.4 (C-5'), 133.6 (C-6'), 163.4 (C-8'), 117.7 (C-8a), 194.4 (C-9'), 183.6 (C-10'), 16.4 (CH3-3').

3.4 In vitro anti-plasmodial assay.

The crude extract and pure compounds were assayed using an automated micro-dilution technique to determine 50% growth inhibition of cultured parasites (Chulay *et al.*, 1983; Desjardins *et al.*, 1979). Two different strains, chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2), of *P. falciparum* were grown in a continuous culture supplemented with mixed gas (90% nitrogen, 5% oxygen, 5% carbon dioxide), 10% human serum, and 6% hematocrit of A+ red blood cells. Once cultures reach a parasitemia of 3% with at least a 70% ring development stage present, parasites were transferred to a 96 well micro-titer plate with wells pre-coated with compound (Desjardin *et al.*, 1979). The samples were serially diluted across the plate to provide a range of concentrations used to determine IC_{50} values. Plates were incubated in a mixed gas incubator for 24 hours. Following the specified incubation time, [^{3}H]-hypoxanthine was added and parasites allowed growing for an additional 18 hours. Cells were processed with a plate harvester (TomTec) onto filter paper and washed to eliminate unincorporated isotope. Filters were then measured for activity in a micro-titer plate scintillation counter (Wallac). Data from the counter was imported into a Microsoft Excel spreadsheet, which is then imported into an oracle database/ program to determine IC_{50} values. A minimum of three separate determinations was carried out for each sample (Desjardin *et al.*, 1979).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

The roots of *Knifophia thomsonii* (Asphodelaceae) was extracted using dichloromethane/methanol (1:1) mixture. After removal of the solvent the extract was tested for anti-plasmodial activity against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. The extract showed significant antiplasmodial activities with IC_{50} values of 3.2 µg/ml (D6) and 6.4 µg/ml (W2).

The extract was subjected to chromatographic separation, which led to the isolation of eleven anthraquinone derivatives (both monomeric and dimeric) and two long alkyl chain substituted benzaldehyde derivatives. The structures of these compounds were determined using 1D (^1H and ^{13}C), 2D (COSY, HMBC and HMQC) NMR and MS and in some cases by direct TLC comparison with authentic sample. The characterization of these compounds is presented below.

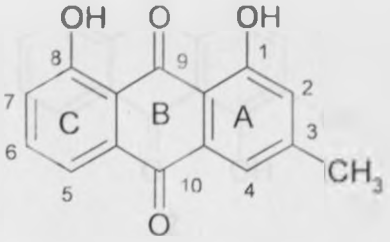
4.1 ANTHRAQUINONE MONOMERS

4.1.1 Chrysophanol (1)

Compound **1** was isolated as yellowish red crystals with a melting point of 195-197°C and an R_f value of 0.50 (5% EtOAc in hexane). The ^1H NMR (Table 2) of this compound showed two downfield shifted hydroxyl protons at δ_H 11.98 and 12.09, which is consistent with a 1,8-dihydroxyanthraquinone skeleton. In support of this, the UV showed absorption bands at λ_{max} 270, 288 and 430 nm which are characteristic of 1,8-dihydroxyanthraquinones.

The ^1H NMR indicated the presence of three mutually coupled aromatic protons with an AMX spin system at δ_H 7.76 (*dd*, *J*=7.5, 1.2 Hz), 7.65 (*t*, *J*=8.1 Hz) and 7.27 (*dd*, *J*=8.0,

1.4 Hz), which are typical of H-5, H-6 and H-7 of mono-substituted (with OH) ring-C of 1, 8-dihydroxyanthraquinones, respectively. The ^{1}H NMR signal at δ_H 2.45 is due to a methyl group attached to an aromatic ring and was placed at C-3 of A-ring on the basis of biogenetic consideration (Wanjohi, 2006). In this ring, the proton at C-4 appeared at δ_H 7.62 (*bd*, *J*=1.0 Hz), while H-2 resonated as a broad doublet at δ_H 7.08 (*J*= 1.0 Hz). The broadening of the signals for H-2 and H-4 resulted from long range coupling (^4J) with the methyl protons. This compound was therefore characterized as 1,8-dihydroxy-3-methylanthraquinone, trivial name chrysophanol (**1**). The identity of this compound was confirmed by TLC comparisons with an authentic sample of chrysophanol (Wanjohi, 2006).



1

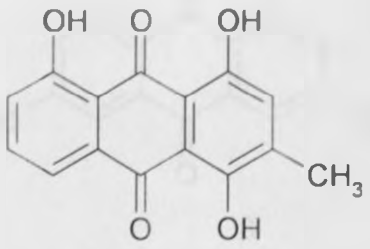
This compound is a common metabolite in the genus *Kniphofia* (Van wyk *et al.*, 1995) and also a constituent of the genera *Cassia*, *Rumex*, *Rheum*, *Asphodelus*, *Muehlenbeckia* and *Monilinia fructicola* spp. It is widely distributed in plants and also found in the marine annelid *Urechis unicinctus*. It is an antimicrobial and a purgative agent (Thomson, 1987).

4.1.2 Islandicin (2)

Compound **2** was isolated as glittering red crystals, melting point 217-219°C. TLC analysis of the compound showed a magenta spot with an R_f value of 0.50 (5% EtOAc in hexane). The spot changed to purple on exposure to NH_3, a characteristic feature of 1,4,8-trihydroxy or 1,5,8-trihydroxyanthraquinones (Wanjohi, 2006).

In support of this, the ^{1}H NMR spectrum (Table 2) showed three chelated hydroxyl protons, δ_H 12.28 (2 OH) and at 13.48. In addition, the presence of an aromatic methyl

group (C-3) was evident from a doublet (J=0.9 Hz) integrating for three protons at δ_H 2.38. Also present were signals for AMX aromatic protons, δ_H 7.88 (*dd*, J= 7.5, 1.1 Hz), 7.69 (*dd*, J=7.5, 0.9 Hz) and 7.30 (*dd*, J=7.5, 1.2 Hz) corresponding to C-ring aromatic protons (C-5, C-6 and C-7 respectively), with C-8 substituted with an hydroxyl group. In A-ring, a quartet observed at δ_H 7.15 (J=0.9 Hz) is due to H-2 having a ^4J coupling with the biogenetically expected methyl group at C-3. The two remaining hydroxyl groups were then placed at C-1 and C-4 of this ring. This compound was therefore identified as 1,4,8-trihydroxy-3-methylanthraquinone, trivial name islandicin (**2**).



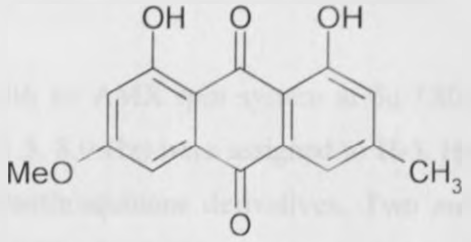
2

The compound has been reported from the stem bark of *Ventilago bombaensis* (Rhamnaceae) (Pepalla *et al.*, 1992); from the stem bark of *Maesopsis eminii* (Rhamnaceae) (Cumming and Thomson, 1970) and from rhizomes of some *Kniphofia* species (Yenesew *et. al.*, 1988).

4.1.3 Physcion (3)

Compound **3** was isolated as a yellow powder with an R_f value of 0.50 (5% EtOAc in hexane). The ^1H NMR (Table 2) of this compound showed two downfield shifted hydroxyl protons at δ_H 12.13 and 12.33, which are consistent with a 1,8-dihydroxyanthraquinone skeleton. The presence of four aromatic protons at (δ_H 7.38, 6.70, 7.64 and 7.09), a methyl (δ_H 2.46) and a methoxyl (δ_H 3.92) groups were also evident from the ^1H NMR spectrum (Table 2).

The methyl is placed at C-3 of ring-A as expected biogenetically. In agreement with this, H-2 and H-4 of this ring appeared at δ_H 7.09 (*bq*, $J \approx 1.7$ Hz) and 7.64 (*bd*, $J = 1.2$ Hz), respectively showing the characteristic *meta*-coupling. These signals also showed long range (^1J) coupling with CH_3-3. In C-ring, the *meta*-coupled protons at δ_H 7.38 (*d*, $J = 2.7$ Hz) and 6.70 (*d*, $J = 2.4$ Hz) were assigned to H-5 and H-7, respectively, with the methoxy group being located at C-6. Therefore, this compound was identified as 1,8-dihyroxy-3-methyl-6-methoxyanthraquinone (trivial name physcion (**3**).



3

This is the first report on the occurrence of C-6 oxygenation in 1,8-dihydroxyanthraquinone in the genus *Kniphofia*. The compound is widely distributed in lichens, e.g. *Parmelia* spp., higher plants, e.g. *Rumex* spp. (Midiwo and Rukunga, 1985) and produced by *Aspergillus* and *Penicillium* spp. It is also isolated from the marine annelid *Urechis unicintus*. Biologically, it is an antimicrobial agent and possesses purgative properties (Ulicky, *et al.*, 1991).

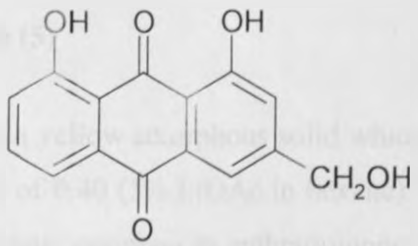
Table 2: ^{1}H NMR (300 MHz) data for compound **1**, **2** and **3** ($CDCl_3$)

Position	Compound		
	1 δ_H *m* (*J* in Hz)	**2** δ_H *m* (*J* in Hz)	**3** δ_H *m* (*J* in Hz)
2	7.08 *bdd* (1.0)	7.15 *bdd* (0.9)	7.09 *bq* (1.7)
3	2.45 *s*	2.38 *d* (0.9)	2.46 *s*
4	7.62 *bdd* (1.0)		7.64 *bd* (1.2)
5	7.76 *dd* (1.2, 7.5)	7.88 *dd* (1.1, 7.5)	7.38 *bd* (2.7)
6	7.65 *t* (8.1)	7.69 *dd* (0.9, 7.5)	3.92 *s*
7	7.27 *dd* (1.4, 8.0)	7.30 *dd* (2.0, 7.5)	6.70 *d* (2.4)
6-OMe			3.92 *s*
1-OH	11.98 *s*	12.28 *s*	12.33 *s*
4-OH		13.48 *s*	
8-OH	12.09 *s*	12.28 *s*	12.13 *s*

4.1.4 Aloe-emodin (4)

Compound **4** was isolated as an orange amorphous solid which on TLC analysis showed a yellow spot with an R_f value of 0.5 (30% EtOAc in hexane). The spot changed to purple on exposure to NH_3, which is typical of 1,8-dihydroxyanthraquinones. In support of this, the UV spectrum showed absorption bands at λ_{max} 271 and 429 nm, with the ^1H and ^{13}C NMR spectra (Table 3) showing two chelated hydroxyl protons at δ_H 12.05 and 12.01 for OH groups at C-1 (δ_C 164.1) and C-8 (δ_C 163.7), an oxymethylene peak at δ_H 4.81 *s* (δ_C 64.4) and carbonyl peaks at δ_C 194.5 (C-9) and 182.9 (C-10).

Three aromatic protons with an AMX spin system at δ_H 7.80 *d* (*J*=1.0, 8.0 Hz), 7.83 *t* (*J*=8.0 Hz) and 7.37 *d* (*J*=1.5, 8.0 Hz) were assigned to H-5, H-6 and H-7 respectively of ring-C of a 1,8-dihydroxyanthraquinone derivatives. Two *meta* coupled protons at δ_H 7.37 (*J*=1.5 Hz, H-2) and 7.80 (*J*=1.0 Hz, H-4) resonated as broad doublets. Instead of the methyl group expected biogenetically at C-3 for anthraquinones (Wanjohi, 2006), there was an oxymethylene peak (δ_H 4.81, *s*) at position 3 of ring A. The broadening of the signals for H-2 and H-4 could have resulted from long range coupling (^5J) with the oxymethylene protons. This compound was therefore characterized as 1,8-dihydroxy-3-hydroxymethyleneanthraquinone trivial name aloe-emodin (**4**).



4

The compound is common in aloes, and has also been reported from, the stem bark of *Cascara sagrada*, *Rhamnus purshiana*, *Rhamnus alaternus*, Chinese rhubarb *Rheum palmatum* and *Rheum undulatum*, *Rumex orientalis* and leaf fruit of *Cassia alata*. It is also found in *Asphodelus microcarpus*, *Asphodelus fistulosus*, *Xanthorrhoea australis* and *Oroxylum indicum* (Dictionary of Natural products, 2008). It is used as a starting material for the synthesis of anthracycline antibiotics and shows some antileukaemic,

antimicrobial and antimutagenic activity as well as antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA). It is used as a purgative and an antiseptic agent (Dictionary of Natural products, 2008).

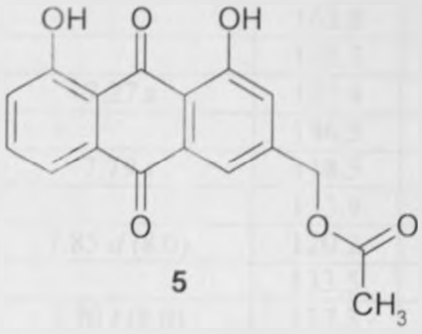
Table 3: ^{1}H (500 MHz), ^{13}C (125 MHz) NMR data along with HMBC correlation for compound **4** (acetone-d_6.)

POSITION	^{1}H δ_H *m* (*J* in Hz)	^{13}C,	HMBC
1		164.1	
1a		117.9	
2	7.37 *bd* (1.5)	122.3	C-1a, C-4
3		155.6	
4	7.80 *bd* (1.0)	120.1	C-1a, C-2
4a		138.1	
5	7.80 *d* (1.0, 8.0)	121.1	C-8a
5a		137.9	
6	7.83 *t* (8.0)	138.9	C-8a, C-5a, C-7
7	7.37 *d* (1.5, 8.0)	125.8	C-8a, C-5
8		163.7	
8a		117.6	
9		194.5	
10		182.9	
3-CH_2OH	4.81	64.4	C-2
1-OH	12.05 *s*		C-1a, C-2
8-OH	12.01 *s*		C-7

4.1.5 Aloe-emodin acetate (5)

Compound **5** was isolated as a yellow amorphous solid which on TLC analysis showed a yellow spot with an R_f value of 0.40 (5% EtOAc in hexane). The spot changed to red on exposure to NH_3, a characteristic common to anthraquinones. In support of this, the UV spectrum showed absorption bands at λ_{max} 268 and 429 nm, while the ^{1}H and ^{13}C NMR spectra (Table 4) showed peaks that were almost similar to aloe-emodin (section **4.1.4**) including the carbonyl peaks at δ_C 192.7 and 181.5 as well as the two hydroxyl singlets at δ_H 12.07 and 12.05. The difference is the presence of a downfield shifted methylene at δ_H 5.19 *brs* (δ_C 64.7), an up-field shifted methyl (acetate methyl) at δ_H 2.19 (δ_C 20.8) and an ester carbonyl at δ_C 170.0. This suggested that compound **5** is an acetate derivative of **4**.

This compound was therefore identified as 3-acetoxymethylene-1,8-dihydroxyanthraquinone, trivial name aloe-emodin acetate (**5**).



5

This is only the third report on the occurrence of this compound in nature having been isolated earlier (Sharma and Rangaswami, 1977) from the roots of *Rumex acetosa* (Polygonaceae) and the leaves of *Kniphofia foliosa* by Berhanu and Dagne in 1984.

Table 4: ^{1}H (500 MHz), ^{13}C (125 MHz) NMR data together with HMBC correlation for compound **5** ($CDCl_3$)

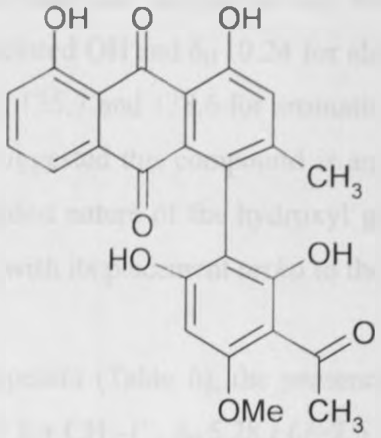
POSITION	^{1}H δ_H *m* (*J* in Hz)	^{13}C	HMBC
1		162.8	
1a		115.3	
2	7.27 *s*	122.4	C-1, C-1a, C-4
3		146.5	
4	7.79 *s*	118.5	C-1a, C-2
4a		133.9	
5	7.85 *d* (8.0)	120.2	C-6, C-7
5a		133.5	
6	7.70 *t* (8.0)	137.3	C-5, C-5a, C-8
7	7.32 *d* (8.0)	124.8	C-5, C-6, C-8a
8		162.6	
8a		115.8	
9		192.7	
10		181.5	
3-C(O)		*170.0	
3-$CH_2OC(O)CH_3$	2.19 *s*	20.8	3-C(O)
3-CH_2OAc	5.19 *brs*	64.7	C-2, C-3, 3-C(O), C-4
1-OH	12.07 *s*		C-2, C-1a
8-OH	12.05 *s*		C-7, C-8a

* From HMBC

4.1.6 Knipholone (6)

Compound **6** was isolated as deep red needles which showed an orange spot on TLC with an R_f value of 0.2 (30% EtOAc in hexane). Comparison of the ^{1}H NMR spectrum (Table 5) of this compound with chrysophanol (**1**) showed that it is a chrysophanol derivative. Thus in the chrysophanol (**1**) part, the two downfield shifted singlet at δ_H 12.60 and 11.99 were due to the chelated hydroxyl groups at C-1 and C-8 whereas the C-ring aromatic protons at H-5, H-6 and H-7 constitute an AMX spin system at δ_H 7.55 *dd* (*J* = 8.0, 1.1 Hz), 7.75 *t* (*J* = 8.0 Hz), and 7.29 *dd* (*J* = 8.0, 2.0 Hz). In the ring A, the signal for H-4 is missing with H-2 appearing as a singlet at δ_H 7.36, while the methyl at C-3 was up-field shifted at δ_H 2.17 relative to that of chrysophanol (**1**). These NMR features suggested that this compound has an aromatic substituent at C-4 on a chrysophanol skeleton.

The substituent at C-4 was identified as acetylphloroglucinol methyl ether from the ^{1}H NMR (δ_H 14.20 for OH-2'; 6.24 (*s*) for H-5'; 2.63 (*s*), 3H, for the acetyl protons at C-3'; 3.98 (*s*), 3H, for OMe at C-4'). Therefore, compound **6** was identified as 4-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-1,8-dihydroxy-3-methylanthraquinone, trivial name knipholone (Bezabih and Abegaz, 1998). The identity was confirmed by direct comparison with an authentic sample (Wanjohi, 2006).



6

Besides being found widely in *Bulbine* and *Kniphofia* species, knipholone has also been reported from the pods of *Senna didymobotrya* (Alemayehu, *et al.*, 1996). Biologically, it has been reported to be a selective inhibitor of leukotriene metabolism (Wube *et al.*, 2006) and is a potent antimalarials compound with an IC_{50} value of 1.49μg/mL (Wube *et al.*, 2005).

Table 5: ^{1}H NMR (200 MHz) chemical shift values for compound **6** ($CDCl_3$)

POSITION	δ_H *m* (*J* Hz)
2	7.36 *s*
5	7.55 *dd* (1.1, 8.0)
6	7.75 *t* (8.0)
7	7.29 *dd* (2.0, 8.0)
5'	6.24 *s*
3-CH_3	2.17 *s*
3'-C(O)-CH_3	2.63 *s*
4'-OCH_3	3.98 *s*
1-OH	12.60 *s*
2'-OH	14.20 *s*
8-OH	11.99 *s*

4.2 BENZALDEHYDE DERIVATIVES

4.2.1 Flavoglaucin (7)

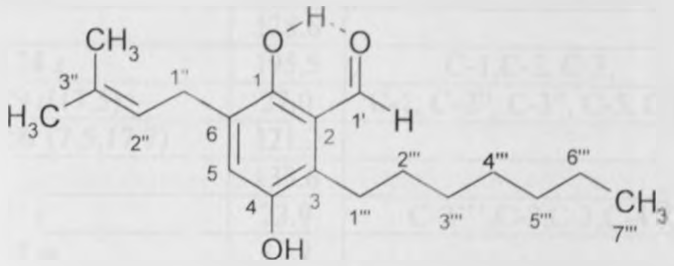
Compound 7 was isolated as yellow crystals (mpt. 105-109°C) with an R_f value of 0.53 (5% EtOAc in hexane). The UV spectrum showed absorption bands at λ_{max} 274 and 391 nm. EIMS analysis showed a molecular ion peak at *m/z* 304 corresponding to $C_{19}H_{28}O_3$. The ^{1}H NMR (δ_H 11.93 for chelated OH and δ_H 10.24 for aldehydic proton) and ^{13}C NMR (δ_C 155.8, 117.3, 128.6, 144.9, 125.7 and 128.6 for aromatic carbons and δ_C 195.5 for the aldehydic carbonyl) spectra suggested this compound is an *ortho*-hydroxylbenzaldehyde derivative. The highly deshielded nature of the hydroxyl group is due to chelation with the carbonyl and is consistent with its placement *ortho* to the aldehyde group.

From the ^{1}H and ^{13}C NMR spectra (Table 6), the presence of a prenyl substituent [δ_H 3.29, *d* (*J*=7.5 Hz) and δ_C 27.0 for CH_2-1"; δ_H 5.28 *t* (*J*=7.5, 17 Hz) and δ_C 121.2 for CH-2"; a quaternary carbon resonating at δ_C 133.6 for C-3"; δ_H 1.76, 1.70 *brs*, and δ_C 17.8, δ_C 25.8 for $(CH_3)_2$ -3"], an alkyl substituent (C_7H_{15}, at δ_H 2.91, *t*; 1.58, *m*; 1.36, *m* and 0.90, *t*); hydroxyl group (δ_H 4.38, *s*; δ_C 144.9) and the only aromatic singlet at δ_H 6.90 is consistent with a tetra-substituted benzaldehyde derivative.

The COSY spectrum showed that the aromatic proton at δ_H 6.90 (H-5) correlated (allylic coupling) with a proton on the prenyl substituent at δ_H 3.29 *d* (CH_2-1") implying the two are adjacent to each other. The HMBC spectrum showed correlation between the aldehydic proton at δ_H 10.24 with C-1 (155.8) (verifying the aldehydic group is actually *ortho*-substituted to the hydroxyl group), and with C-2 (117.3) and C-3 (128.6) of the benzene ring. HMBC correlation was also observed between the methylene protons of the prenyl substituent at δ_H 3.29 (CH_2-1") with C-6 (128.57), C-1 (155.8) and C-5 (125.6) giving evidence that the prenyl substituent is actually attached to the benzene ring at the C-6 position.

In addition, the chelated hydroxyl group at δ_H 11.93 showed HMBC correlation to C-2 (117.3), C-3 (128.6) and C-6 (128.57) of the aromatic ring. Correlations were also noted

between aromatic proton (H-5, δ_H 6.90) with C-1 (155.8), C-4 (144.9) and C-6 (128.57) for the aromatic ring and also with the protons on the prenyl substituent (CH_2-1"). The methylene protons (CH_2-1"") of the long alkyl chain at δ_H 2.91 *t* correlated with C-2, C-3 and C-4 of the aromatic ring confirming the point of attachment of the chain to the ring is at C-3. These spectroscopic data confirm that this compound has structure 7, trivial name flavoglaucin.



7

This compound has been isolated from *Aspergillus flavus* and other *Aspergillus* spp. and a marine derived *Microsporum* spp. It is biologically used as a mycotoxin (antifungal agent) (Dictionary of Natural products, 2008). This appears to be the first report on the occurrence of this compound in higher plants.

Table 6: ^{1}H (300 MHz), ^{13}C (75 MHz) NMR together with H,H-COSY and HMBC data for compound 7 ($CDCl_3$)

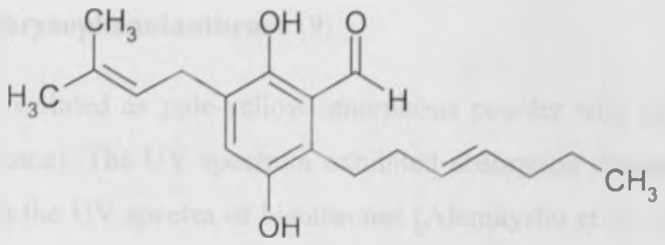
POSITION	^{1}H δ_H *m* (*J* in Hz)	^{13}C	HMBC (^{2}J, ^{3}J)	H,H-COSY
1		155.8		
2		117.3		
3		128.6		
4		144.9		
5	6.90 *s*	125.6	C-1, C-1", C-3, C-4,	CH_2-1"
6		128.6		
1'	10.24 *s*	195.5	C-1,C-2, C-3,	
1"	3.29 *d* (7.5)	27.0	C-1, C-2", C-3", C-5, C-6	$(CH_3)_2$-3"
2"	5.28*t* (7.5,17.7)	121.2		CH_2-1", $(CH_3)_2$-3"
3"		133.6		
1'''	2.91 *t*	23.9	C-2''',C-2,C-3,C-4	CH_2-2'''
2'''	1.58 *m*	31.9		
3'''		29.6		
4'''	1.36 *m*	29.1		CH_2-1'''
5'''		31.9		
6'''		22.6		
7'''	0.90 *t*	14.1	C-6''', C-5''',	
3"-CH_3	1.76 *brs*	17.8	C-2", C-3"	CH_2-1", CH_2-2"
3"-CH_3	1.70 *brs*	25.8	C-2", C-3"	CH_2-1", CH_2-2"
1-OH	11.93 *s*		C-2, C-3	
4-OH	4.38 *s*			

4.2.2 3''',4'''-Dehydroflavoglaucin (8)

Compound **8** was isolated as a yellow amorphous solid with an R_f value of 0.46 (5% EtOAc in hexane). The ^{1}H NMR data for **8** was indicative of a benzaldehyde skeleton. The ^{1}H NMR spectrum of **8** was similar to that of compound **7** (Section 5.2.1) suggesting identical skeleton with the only difference being on the nature of the long alkyl chain substituent.

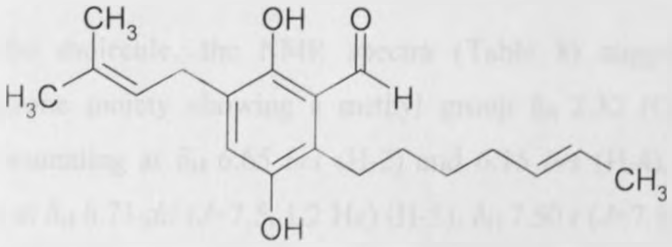
In the ^{1}H NMR spectrum of compound **8**, the presence of a double bond between C-3''' and C-4'''was evident from the two olefinic methine protons appearing more or less at the same frequency (due to pseudo-symmetry) as a triplet (at δ_H 5.51) placed in the double bond at C-3'''. Other peaks for the straight chain were; δ_H 2.96 *t*: 2H (C-1'''), 2.28 *bq*; 2H (C-2'''), 1.99 *bq*; 2H (C-5'''). 1.34 *m*: 2H (C-6''') and 0.87 *t*: 3H (C-7''')

while the rest were similar to the ones observed for compound **7** (**Table 3**). Therefore compound **8** was characterized as 3-(3-heptene)-1,4-dihydroxy-6-prenylbenzaldehyde.



8

In the spectrum where compound **8** is the major compound, another set of ^{1}H NMR signals similar to those of **8** were observed. These additional peaks could be due to an additional compound in the sample with the only difference from compound **8** being on the position of the double bond on the alkene chain, which in this case is at C-5''' (5''',6'''-dehydroflavoglaucin **8a**). (**Table 7**)



8a

Table 7: ^{1}H NMR (300 MHz) data ($CDCl_3$) for compound **8** and **8a**

POSITION	**8**, ^{1}H δ_H *m* (*J* in Hz)	**8a**, ^{1}H δ_H *m* (*J* in Hz)
5	6.90 *s*	6.89 *s*
1'	10.23 *s*	10.24
1''	3.30 *d* (7.2)	3.30 *d* (7.2)
2''	5.28 *t* (7.2,17.4)	5.28 *t* (7.2,17.4)
1'''	2.96 *t*	2.88 *t*
2'''	2.28 *bq*	2.28 *bq*
3'''	5.51 *t*	1.31 *bq*
4'''		2.04 *bq*
5'''	1.99 *bq*	4.23 *bq*
6'''	1.34 *m*	5.41 *bq*
7'''	0.87 *t*	1.64 *d*
3''-CH_3	1.76 *s*	1.76 *s*
3''-CH_3	1.70 *s*	1.70 *s*
1-OH	11.95 *s*	11.93 *s*
4-OH	4.38	4.38

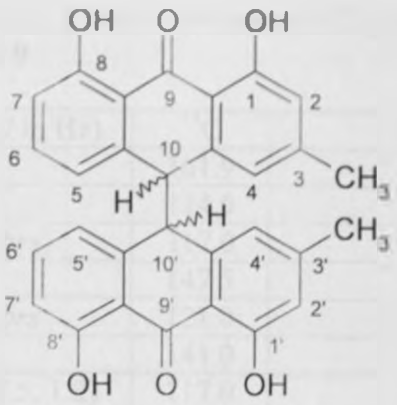
4.3 ANTHRAQUINONE DIMERS

4.3.1 10,10'-Bichrysophanolanthrone (9)

Compound **9** was isolated as pale-yellow amorphous powder with an R_f value of 0.36 (5% EtOAc in hexane). The UV spectrum exhibited absorption maxima at 271 and 367 nm consistent with the UV spectra of bianthrones (Alemayehu et al., 1992). The HRMS ($[M+1]^+$ at *m/z* 479.1483, $C_{30}H_{23}O_6$), together with the ^1H and ^{13}C NMR spectra, [showing signals arising from two aromatic methyl groups (δ_H 2.22 and 2.32; δ_C 21.9 and 22.1), two sp^3 hybridized methine groups (δ_H 4.45 (*J*=1.5 Hz; δ_C 56.3 and δ_H 4.49 (*J*=1.5 Hz; δ_C 56.3)), four chelated hydroxyl groups (δ_H 11.71; δ_C 161.9, δ_H 11.88; δ_C 162.1, δ_H 11.63; δ_C 161.9 and δ_H 11.79; δ_C 162.2) and two carbonyl groups (both at δ_C 191.6)] were consistent with this compound being a bianthrone derivative.

In one half of the molecule, the NMR spectra (Table 8) suggested that it is a chrysophanol anthrone moiety showing a methyl group δ_H 2.32 (CH_3-3), two *meta*-oriented protons resonating at δ_H 6.65 *brs* (H-2) and 6.15 *brs* (H-4), together with an AMX spin system at δ_H 6.73 *dd* (*J*=7.5, 1.2 Hz) (H-5), δ_H 7.50 *t* (*J*=7.5 Hz) (H-6) and δ_H 6.94 *dd* (*J*=7.5, 1.2 Hz) (H-7). The signals attributed to H-4 (δ_H 6.15) and H-5 (δ_H 6.73) were relatively up-field compared to what is observed in chrysophanol (**1**), supporting the anthrone nature of this moiety in compound **9**. In agreement with this, the ^1H and ^{13}C NMR showed signals of a methine group at position 10 (δ_H 4.49; δ_C 56.3) instead of a carbonyl as in anthraquinone such as chrysophanol (**1**). The methine signal (δ_H 4.49) appeared as a doublet (*J*=1.5 Hz) suggesting that it is coupled with similar methine proton (CH-10') from the other half of the molecule. In addition, the HMBC spectrum showed correlation of H-10 with C-4a, C-4, C-4a', C-5a' and C-8a (Table 8) signifying that indeed the point of linkage was at 10/10' of the two chrysophanol moieties.

In fact the MS ($[M+1]^+$ at *m/z* 479.1483, $C_{30}H_{22}O_6$ and the fragment at *m/z* 239) and NMR (Table 8) showing identical pattern for the other half of the molecule, is in agreement with the other half of the molecule being a chrysophanol-anthrone linked at C-10'. Therefore this compound was characterized as 10,10'-bichrysophanolanthrone (**9**).



9

This compound has been isolated from *in vitro* cultures of *Cassia didymobotrya* (Leguminosae, Caesalpinoideae) (Monache *et al.*, 1990), from the seeds of *Cassia obstusifolia*, from the heartwood of *C. garrettina* and also from the leaves of *Senna longiracemosa* (Alemayehu *et al.*, 1992). However this appears to be the first report on its occurrence in the family Asphodelaceae.

Table 8: ^{1}H (500 MHz), ^{13}C (125 MHz) NMR data ($CDCl_3$) along with HMBC correlation for compound **9**

POSITION	^{1}H δ_H *m* (*J* in Hz)	^{13}C	HMBC
1		161.9	
1a		114.0	
2	6.65 *brs*	117.0	C-1a, CH -3, C-4
3		147.5	
4	6.15 *brs*	121.0	C-1a, C-2
4a		141.0	
5	6.73 *dd* (7.5, 1.2)	117.0	C-7
5a		142.1	
6	7.50 *t* (7.5)	135.7	C-5a, C-8
7	6.94 *dd* (7.5, 1.2)	116.8	C-5
8		162.1	
8a		117.2	
9		191.6	
10	4.49 *d* (1.5)	56.3	C-1a, C-4a, C-4, C-4a', C-5a, C-5a', C-8a
1'		161.9	
1a'		114.5	
2'	6.65 *brs*	116.9	C-1a', CH_3-3', C-4'
3'		147.3	
4'	5.73 *brs*	121.2	C-1a', C-2'
4a'		140.9	
5'	6.33 *d* (8.0)	119.4	C-7',
5a'		142.1	
6'	7.30*t* (8.0)	135.3	C-8a', C-8'
7'	6.87 *d* (8.0)	116.9	C-5'
8'		162.2	
8a'		116.7	
9'		191.6	
10'	4.45 *d* (1.5)	56.3	C-1a', C-4a', C-4', C-4a, C-5a, C-5a', C-8a'
3-CH_3	2.32 *s*	22.1	C-2, C-3, C-4
3'-CH_3	2.22 *s*	21.9	C-2', C-4', C-3'
1-OH	11.71 *s*		C-1a, C-2
1'-OH	11.63 *s*		C-1a', C-2'
8-OH	11.88 *s*		C-7
8'-OH	11.79 *s*		C-7'

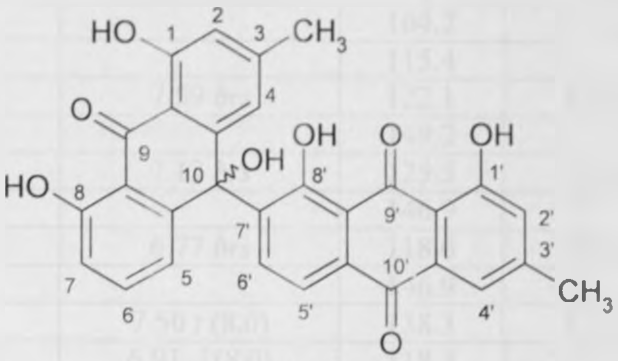
4.3.2 10-Hydroxy-10-(chrysophanol-7'-yl)-chrysophanol anthrone (10)

Compound **10** was isolated as an orange amorphous powder which turned purple on exposure to ammonia, R_f value of 0.32 (20% EtOAc in hexane). The HRMS (M^+ at *m/z* 508.1194, $C_{30}H_{20}O_8$) and the ^1H and ^{13}C NMR (Table 9) along with the UV (λ_{max} 271, 292, 389 and 434 nm) are indicative of an anthrone-anthraquinone dimer. That **10** might be a dimer based on two chrysophanol moieties was deduced from the presence of two aromatic methyl signals (at δ_H 2.23 and 2.45), four chelated hydroxyl signals (at δ_H 12.24, 12.26, 12.32 and 12.34) along with the corresponding oxygenated aromatic carbon atoms (δ_C 164.2, 164.2, 163.9, 163.7) as well as three carbonyl groups (at δ_C 202.0, 194.9 and 182.8).

In one half of the molecule, the NMR signals were comparable to what was observed for chrysophanol (**1**) (section **5.1.1**) with methyl group (δ_H 2.23) located at C-3, two *meta* protons assignable to H-2 and H-4, (resonating at δ_H 7.59 *brs* and 7.12 *brs*, respectively), together with an AMX spin pattern (at δ_H 6.77 *brs*, δ_H 7.50 *t* (*J*=8.0 Hz) and δ_H 6.91 *d* (*J*=8.0 Hz)) which could be assigned to H-5, H-6 and H-7 of the chrysophanol moiety, respectively. Both H-4 (δ_H 7.12) and H-5 (δ_H 6.77) are shielded compared to what is observed for these protons in chrysophanol (**1**) (δ_H 7.62 and 7.76, respectively) suggesting that C-10 in this half of the molecule is not a carbonyl. The presence of an sp^3 hybridized oxygenated carbon at δ_C 75.1 indicated the oxanthrone nature of this half of the molecule and that C-10 is the position of attachment to the other half of the molecule.

For the other half of the molecule, a similar pattern was observed with the ^1H NMR showing a methyl group at δ_H 2.45 *s* (C-3'), two *meta*-coupled protons assignable to H-2' and H-4' (resonating at δ_H 6.77 *brs* and 6.88 *brs* respectively), whereas H-5' and H-6' resonated as *ortho*-coupled protons at δ_H 7.98 *brs* and 8.81 *brs* respectively. In comparison with chrysophanol (**1**), the replacement of an AMX spin system with this AX pattern in ring C for this half of the molecule showed that C-7' is the point of attachment to the other half. Therefore this compound is a dimer where chrysophanol anthrone is

coupled at C-10 to C-7' of a chrysophanol moiety (10-Hydroxy-10-(chrysophanol-7'-yl) - chrysophanol anthrone **10**).



10

This compound has been reported from *Kniphofia foliosa* (Wube *et al.*, 2005), from the rhizomes of *Aloe saponaria* and *Senna longiracemosa* (Alemayehu *et al.* 1992). The antiplasmodial activity (against the chloroquine sensitive 3D7 strain of *P. falciparum* with an IC_{50} value of 0.260 µg/mL) and a weak cytotoxic activity against KB (human epidermoid carcinoma) cell line with an ED_{50} value of 104 µg/mL has been reported for this compound (Wube *et al.*, 2005).

Table 9: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data (acetone-d_6) along with HMBC correlations for compound **10**

POSITION	^{1}H δ_H *m* (*J* in Hz)	^{13}C	HMBC
1		164.2	C-1a
1a		115.4	
2	7.59 *brs*	122.1	C-1a, C-4
3		149.2	
4	7.12 *brs*	125.5	C-1a, C-2
4a		146.9	
5	6.77 *brs*	118.6	C-7, C-8a
5a		146.9	
6	7.50 *t* (8.0)	138.3	C-5a, C-8
7	6.91 *d* (8.0)	118.3	C-5, C-8a
8		164.2	C-7
8a		116.1	
9		194.9	
10		75.1	
1'		163.9	C-1a
1a'		114.0	
2'	6.77 *brs*	122.3	C-1a', C-4
3'		150.3	
4'	6.88 *brs*	120.9	C-2'
4a'		132.7	
5'	7.98 *brs*	120.5	C-8a'
5a'		132.7	
6'	8.81 *brs*	135.1	
7'		130.3	
8'		163.7	
8a'		116.1	
9'		182.8	
10'		202.0	
3-CH_3	2.23 *s*	22.79	C-2, C-3, C-4
3'-CH_3	2.45 *s*	22.75	C-2', C-3', C-4'
1-OH	12.24 *s*		
1'-OH	12.32 *s*		
8-OH	12.26 *s*		
8'-OH	12.34 *s*		
10-OH	5.6 *s*		

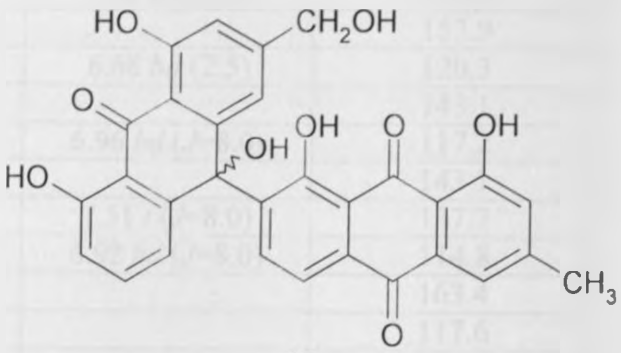
4.3.3 10-Hydroxy-10-(chrysophanol-7'-yl)-aloe-emodin anthrone (11)

Compound **11** was isolated as a yellow solid with an R_f value of 0.40 in 40% EtOAc in hexane. The HRMS (M^+ at m/z 523.5003, $C_{30}H_{20}O_9$), the ^1H and ^{13}C NMR (Table 10) along with the UV (λ_{max} 271, 292, 385 and 494 nm), were indicative of an anthrone-anthraquinone dimer. The ^1H NMR spectrum of compound **11** is comparable to that of compound **10** (Section **5.3.2**). However, the ^1H and ^{13}C NMR spectra of **11** showed an oxymethylene peak at (δ_H 4.56; δ_C 63.83) and a methyl at (δ_H 2.46 *brs*; δ_C 22.06) while compound **10** showed two methyl groups. The rest of the peaks including four chelated and one unchelated hydroxyl groups (δ_H 12.29 *s*, 12.30 *s*, 12.32 *s*, 12.34 *s* and 5.91 *s*) along with the corresponding oxygenated aromatic carbon atoms (δ_C 179.26, 163.36, 163.74 and 163.03) and three carbonyl resonances (at δ_C 202.60, 182.12 and 194.36) were similar with small variation in the values.

In one half of the molecule, the ^1H NMR features were similar to those of compound **4** (Section **5.1.4**) including two *meta*-coupled broad doublets for H-2 and H-4 [at δ_H 6.96 (J=1.0 Hz) and 6.68 (J=2.5 Hz), respectively], an AMX spin pattern for H-5, H-6 and H-7 [at δ_H 6.96 *bd* (J=8.0 Hz), δ_H 7.51 *t* (J=8.0 Hz) and 6.92 *bd* (J=8.0 Hz), respectively] and an oxymethylene peak resonating as a broad singlet at δ_H 4.56 (C-3). The signals attributed to H-4 (δ_H 6.68) and H-5 (δ_H 6.96) were relatively shielded (due to the lack of anisotropic effect from the C=O group) compared to what is observed for these protons in aloe-emodin (**4**) (δ_H 7.80 and 7.80, respectively) suggesting that C-10 in this half of the molecule is not a carbonyl. The presence of an sp^3 hybridized oxygenated carbon indicated the aloe-emodinoxanthrone nature of this half of the molecule and that C-10 is the position of attachment to the other half of the molecule.

On the other hand, the second half of the molecule exhibited values similar to chrysophanol (**1**) with coupling patterns among the aromatic protons indicating a broad singlet methyl proton at δ_H 2.46 which is assignable to Me-3', two *meta*-coupled protons assignable to H-2' and H-4' resonating at δ_H 7.58 *s* and 7.11 *s* ,respectively and two *ortho*-coupled doublets (at δ_H 7.98 (J=7.6 Hz) and 8.81 (J=7.6 Hz)) which could be

assigned to H-5' and H-6' respectively. The de-shielded nature of the *ortho*-coupled protons and the absence of a signal for H-7', signified that this was the position of linkage between the two molecules. This compound was thus characterised as 10-hydroxy-10-(chrysophanol-7'-yl)-aloe-emodin anthrone (common name, chrysalodin).



11

This compound has been reported from some *Kniphofia foliosa* (Dagne, *et al.*, 1987). The compound showed borderline cytotoxic activity against *in vitro* growth of KB tissue culture cells with an ED_{50} value of 10μg/ml (Dagne, *et al.*, 1987).

Table 10: ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C (acetone-d_6, 125 MHz) NMR data for compound **11**

POSITION	^{1}H δ_H *m* (*J* in Hz)	^{13}C
1		163.5
1a		116.7
2	6.96 *bd* (1.0)	114.6
3		153.9
4	6.68 *bd* (2.5)	120.3
4a		143.1
5	6.96 *bd* (*J*=8.0)	117.2
5a		143.1
6	7.51 *t* (*J*=8.0)	137.7
7	6.92 *bd* (*J*=8.0)	114.8
8		163.4
8a		117.6
9		194.4
10		*
1'		163.0
1a'		114.1
2'	7.58 *s*	124.8
3'		143.1
4'	7.11 *s*	117.9
4a'		134.0
5'	7.98 *d* (*J*=7.6)	120.3
5a'		134.4
6'	8.81 *d* (*J*=7.6)	133.8
7'		121.5
8'		160.5
8a		117.5
9'		182.1
10'		179.3
3-(CH_2OH)	4.56 *brs*	63.8
3'-(CH_3)	2.46 *brs*	22.1
1-OH	12.29 *s*	
1'-OH	12.32 *s*	
8-OH	12.30 *s*	
8'-OH	12.34 *s*	
10-OH	5.91 *s*	

* Not observed

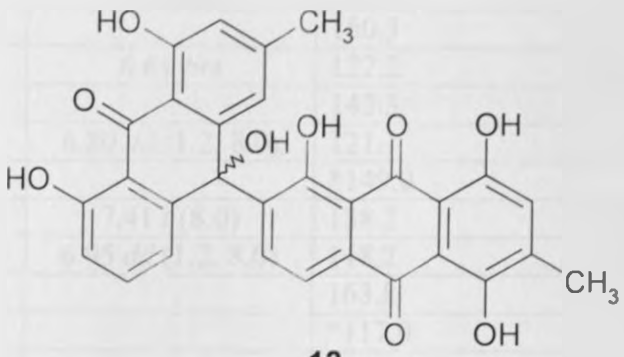
4.3.4 10-Hydroxy-10-(islandicin-7'-yl)-chrysophanolanthrone (12)

Compound **12** was isolated as a red powder with an R_f value of 0.40 in 20% EtOAc in hexane. The HRMS (M^+ at *m/z* 524.1125, $C_{30}H_{20}O_9$), ^1H and ^{13}C NMR (Table 11) along with the UV (λ_{max} 271, 299, 381 and 495 nm) were indicative of an anthrone-anthraquinone dimer. Accordingly, the NMR spectra exhibited two aromatic methyl (δ_H 2.26, δ_C 24.4 and δ_H 2.35, δ_C 24.1), five chelated hydroxyl groups (δ_H 12.08, 12.32, 12.34, 12.44 and δ_H 13.56; δ_C) as well as the corresponding oxygenated carbon atoms (δ_C 163.5, 163.8, 158.9, 160.0 and 158.9 respectively) and three carbonyl groups (δ_C 195.0, 188.2 and 188.2). The presence of three carbonyl groups supported that compound **12** is an anthrone-anthraquinone dimer. The EIMS fragment ion at *m/z* 256 and the molecular ion (M^+ at *m/z* 524.1125) indicated that indeed the compound is a dimer composed of monomers related to chrysophanol and islandicin.

The ^1H NMR spectrum (Table 11) is in agreement that one half of the molecule is chrysophanol-anthrone moiety showing a methyl group (δ_H 2.26) located at C-3, two *meta*-coupled aromatic protons assignable to H-2 and H-4, resonating at δ_H 6.78 (*brs*) and 6.61 (*brs*) respectively, together with an AMX spin system at δ_H 6.80 *dd* (*J*= 8.0, 1.2 Hz), 7.41 *t* (*J*=8.0 Hz) and 6.95 *dd* (*J*=8.0, 1.2 Hz) which could be assigned to H-5, H-6 and H-7 of the chrysophanol-anthrone moiety respectively. The ^{13}C NMR data (δ_C 79.5 for C-10) and the fragment ion at *m/z* 256 indicated the oxanthrone nature of this moiety. The signals attributed to H-4 (δ_H 6.61) and H-5 (δ_H 6.80) are shielded compared to what is observed for these protons in chrysophanol (**1**) (δ_H 7.62 and 7.76, respectively) supporting that C-10 in this half of the molecule is not a carbonyl and the oxanthrone nature of this half of the molecule. Furthermore, the multiplicity (singlet) and the chemical shift position of C-10 (δ_C 79.5) indicated that this is the position of attachment to the other half of the molecule.

For the other half of the molecule, the ^1H NMR spectrum showed a singlet at δ_H 7.10 (H-2') and two *ortho*-coupled protons at δ_H 8.06 *d* (*J*=8.0 Hz) and 8.66 *d* (*J*=8.0 Hz) corresponding to H-5' and H-6' respectively. These data were consistent with this half

being islandicin (3). The de-shielded nature of the *ortho*-coupled protons (δ_H 8.06 and 8.66) and the absence of a signal for H-7', signified that C-7' is the position of linkage in this half of the molecule. Thus compound **12** was identified as 10-hydroxy-10-(islandicin-7'-yl)-chrysophanol anthrone (trivial name chryslandicin).



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This compound has been reported from *Kniphofia foliosa* (Dagne, *et al*, 1987). Mild cytotoxic activity against *in vitro* growth of KB tissue culture cells with an ED_{50} value of 20μg/ml (Dagne, *et al.*, 1987) and a strong antiplasmodial activity against the chloroquine sensitive 3D7 strain of *P. falciparum* with an IC_{50} value of 0.537μg/mL have been reported for this compound (Wube *et al.*, 2005).

Table 11: ^{1}H ($CDCl_3$, 300 MHz). ^{13}C NMR (acetone-d_6+MeOH-d_4, 125 MHz) data along with HMBC correlation for compound **12**

POSITION	^{1}H δ_H *m* (*J* in Hz)	^{13}C	HMBC
1		163.5	
1a		114.2	
2	6.78 *brs*	118.5	C-1a, 3-CH_3, C-4
3		150.3	
4	6.61 *brs*	122.2	C-1a, C-2
4a		143.3	
5	6.80 *dd* (1.2, 8.0)	121.1	C-7, C-8a
5a		*149.0	
6	7.41 *t* (8.0)	138.2	C-5a, C-8
7	6.95 *dd* (1.2, 8.0)	118.2	C-5, C-8a
8		163.8	
8a		*117.0	
9		195.0	
10		79.5	
1'		158.9	
1a'		112.5	
2'	7.10 *s*	130.4	C-1'
3'		149.5	
4'		158.9	
4a'		112.5	
5'	8.06 *d* (8.0)	120.4	C-8a'
5a'		134.6	
6'	8.66 *d* (8.0)	134.4	C-5a, 8'
7'		**	
8'		*160.0	
8a'		*118.0	
9'		188.2	
10'		188.2	
3-CH_3	2.26 *s*	24.4	C-2, C-3, C-4
3'-CH_3	2.35 *s*	24.1	C-2', C-3', C-4',
1-OH	12.08 *s*		
1'-OH	12.34 *s*		
4'-OH	13.56 *s*		
8-OH	12.32 *s*		
8'-OH	12.44 *s*		
10-OH	6.61 *s*		

*Detected from HMBC spectrum; ** Not Detected

4.3.5 10-Hydroxy-10-(islandicin-7'-yl)-aloe-emodinanthrone (13)

Compound **13** was isolated as a red solid with an R_f value of 0.47 in 40% EtOAc in hexane. The HRMS (M^+ at *m/z* 540.3872, $C_{30}H_{20}O_{10}$) and the ^1H NMR spectrum (showing five chelated and one unchelated hydroxyl groups, δ_H 12.29, 12.31, 12.32, 12.34, 13.51 and 3.98) suggested a dimeric anthraquinone derivative composed of 1,8-dihydroxy and 1,4,8-trihydroxyanthraquinone monomers. In agreement with this, the UV spectrum showed absorption bands for both 1,8-dihydroxyanthrone and 1,4,8-trihydroxyanthraquinone chromophores (at λ_{max} 271, 298, 381 and 495 nm). In the ^{13}C NMR, the presence of five oxygenated sp^2 hybridized carbon atoms (Table 12) is in agreement with such skeleton. Comparison of the ^1H and ^{13}C NMR spectra of **13** with those of compounds **2** (Table 2) and **4** (Table 3), showed that one half of the molecule is aloe-emodin (**4**) coupled through C-10 to C-7' of islandicin (**2**).

In agreement with this, the NMR spectra for the first half showed the presence of signals for oxymethylene (δ_H 4.56 *bs*, δ_C 63.8), two chelated and one unchelated hydroxyl groups (δ_H 12.29, δ_C 169.3; δ_H 12.31, δ_C 163.4 and δ_H 6.05), an AMX spin pattern (δ_H 6.94 *bd* (*J*=8.5Hz), 7.51 *t* (*J*=8.0 Hz) and 6.92 *bd* (*J*=8.5 Hz) for H-5, H-6 and H-7 respectively) and *meta*-coupled protons (δ_H 6.95 *brs* and 6.90 *brs* for H-2 and H-4). As in the other dimers the point of attachment is C-10.

For the other half of the molecule, the NMR (Table 12) spectra displayed signals for three chelated hydroxyl groups (δ_H 12.33, δ_C 165.9; δ_H 12.34, δ_C 163.4 and δ_H 13.51, δ_C 159.7 for OH-1', OH-8' and OH-4', respectively), two *ortho*-coupled aromatic protons (δ_H 8.06 *brs* and 8.83 *brs* for H-5' and H-6'), aromatic methyl group (δ_H 2.28 *brs*; δ_C 16.4 for CH_3-3') and an aromatic methine (as a broad singlet at δ_H 7.21 due to H-2' having a ^4J coupling with the biogenetically expected methyl group at C-3'). This half is therefore the islandicin moiety (compound **2**, Section 5.1.2). The AX spin pattern for Ring-C and the absence of H-7', suggested the point of attachment is at C-7'. Thus, compound **13**

was characterized as 10-hydroxy-10-(islandicin-7'-yl)-aloe-emodin anthrone. This compound appears to be novel.

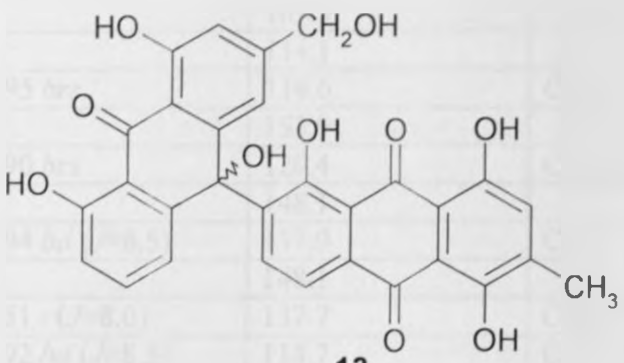

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Table 12: ^{1}H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz) NMR (acetone-d_6) data along with HMBC correlation for compound **13**

POSITION	^{1}H δ_H *m* (*J* in Hz)	^{13}C,	HMBC
1		169.3	
1a		114.1	
2	6.95 *brs*	114.6	C-1a, C-4
3		153.9	
4	6.90 *brs*	120.4	C-1a, C-2
4a		148.1	
5	6.94 *bd* (*J*=8.5)	117.9	C-7
5a		148.1	
6	7.51 *t* (*J*=8.0)	137.7	C-5a, C-8
7	6.92 *bd* (*J*=8.5)	114.7	C-5, C-8a
8		163.0	
8a		115.5	
9		202.8	
10		*	
1'		165.9	
1a'		111.7	
2'	7.21 *brs*	129.9	C-1a', C-4'
3'		144.0	
4'		159.7	
4a'		112.6	
5'	8.06 *brs*	120.4	
5a'		133.8	
6'	8.83 *brs*	133.6	
7'		*	
8'		163.4	
8a'		117.7	
9'		194.4	
10'		183.6	
3-(CH_2OH)	4.56 *brs*	63.8	C-2, C-3, C-4
3'-(CH_3)	2.28 *s*	16.4	C-2',C-3',C-4'
1-OH	12.29 *s*		
1'-OH	12.33 *s*		
4'-OH	13.51 *s*		
8-OH	12.31 *s*		
8'-OH	12.34 *s*		
10-OH	6.05 *s*		

* Not detected

4.4 CHEMOTAXONOMIC SIGNIFICANCE

From the roots of *Kniphofia thomsonii*, this work has resulted in the isolation of five monomeric anthraquinones, one phenylanthraquinone, two benzaldehyde derivatives and five dimeric anthraquinones. Most of the monomeric anthraquinones isolated here are derivatives of chrysophanol (**1**) and lacks oxygenation at C-6 which is typical in the family Asphodelaceae. When additional oxygenation occurs it is on the methyl carbon, as in aloe-emodin (**4**) and aloe-emodin acetate (**5**). Whereas chrysophanol (**1**) and aloe-emodin (**4**) are common in many families [in aloes and has also been reported from the stem bark of *Cascara sagrada*, *Rhamnus purshiana*, *Rhamnus alaternus*, Chinese rhubarb *Rheum palmatum* and *Rheum undulatum*. *Rumex orientalis* and leaf fruit of *Cassia alata*, and also found in *Asphodelus microcarpus*, *Asphodelus fistulosus*, *Xanthorrhoea australis* and *Oroxylum indicum* (Dictionary of Natural products, 2008)], this is only the third report on the occurrence of aloe-emodin acetate in nature having been isolated earlier from the roots of *Rumex acetosa* (Sharma and Rangaswani, 1977) and the leaves of *Kniphofia foliosa* (Berhanu and Dagne, 1984).

The C-6 oxygenated anthraquinone physcion (**3**) has been isolated from this plant and this is the first concrete report the occurrence of C-6 oxygenated 1.8-dihydroxyanthraquinone in the family Asphodelaceae. This compound is widely distributed in lichens, e.g. *Parmelia* spp., higher plants, e.g. *Rumex* spp. (Midiwo and Rukunga, 1985) and produced by *Aspergillus* and *Penicillium* spp. It is also isolated from the marine annelid *Urechis unicintus* (Ulicky, *et al.*, 1991). Islandicin (**2**) is an example of 1.4.8-trihydroxy-3-methyl anthraquinone and has been isolated from the stem bark of *Ventilago bombaensis* (Rhamnaceae) (Pepalla *et al.*, 1992); from the stem bark of *Maesopsis eminii* (Rhamnaceae) (Cumming and Thomson, 1970) and from rhizomes of some *Kniphofia* species (Yenesew *et. al.*, 1988).

The unique compound knipholone (**6**) in which an acetylphloroglucinol methyl ether unit (**30**) is attached to a chrysophanol (**1**) moiety, also occurs in this plant. Comparative studies on the roots of some 14 *Kniphofia* species showed this compound to be the major

pigment in these taxa. It was therefore suggested that compound **6** may be a marker for the genus *Kniphofia* (Van Wyk *et al.*, 1995). Knipholone-type compounds appear to be characteristic constituents for the genera *Kniphofia* (Dagne and Yenesew, 1994), *Bulbinella* (Van Wyk *et al.*, 1995), and *Bulbine* (Bezabih and Abegaz, 1998).

The root of this plant is a rich source of dimeric anthraquinones. Some dimeric anthraquinones have been reported from the leaves of some *Kniphofia* species but their occurrence in the roots is uncommon. These dimers are principally derived from chrysophanol. Two of these dimers are composed of two chrysophanol monomers vis 10,10'-bichrysophanolanthrone (**9**) and 10-hydroxy-10-(chrysophanol-7'-yl)-chrysophanol anthrone (**10**). The latter compound has been reported from *Kniphofia foliosa* (Wube *et al.*, 2005), from the rhizomes of *Aloe saponaria* and *Senna longiracemosa* (Alemayehu *et al.*, 1992); whereas the former compound has been isolated from *in vitro* cultures of *Cassia didymobotrya* (Leguminosae, Caesalpinoideae) (Monache *et al.*, 1990), from the seeds of *Cassia obstusifolia*, from the heartwood of *C. garrettina* and also from the leaves of *Senna longiracemosa* (Alemayehu *et al.*, 1992). This however appears to be the first report on the occurrence of compound **9** in the family Asphodelaceae. The other two dimmers, 10-hydroxy-10-(chrysophanol-7'-yl)-aloe-emodin anthrone (**11**) and 10-hydroxy-10-(islandicin-7'-yl)-chrysophanol anthrone (**12**) are composed of chrysophanol/aloe-emodin and chrysophanol/islandicin monomers respectively. Both compounds have been reported from some *Kniphofia* species (Dagne, *et al.*, 1987). The only new compound isolated here is 10-hydroxy-10-(islandicin-7'-yl)-aloe-emodin anthrone (**13**) which is composed of islandicin/aloe-emodin monomers. In most of the dimers reported in the Asphodelaceae, the linkage is between C-10 of one unit to C-7' of another unit.

This appears to be the first report on the occurrence of the benzaldehyde derivative flavoglaucin (**7**) in higher plants. Normally for such compounds with *para* hydroxyl groups, the most stable structure is the quinonoid form which is not the case here. This is partly because the hydroxyl and the aldehyde group are chelated thereby forming a stable structure that does not easily convert to the otherwise stable quinonoid form. This

compound has been isolated from *Aspergillus flavus* and other *Aspergillus* spp. and a marine derived *Microsporum* spp. It is biologically used as a mycotoxin (antifungal agent) (Dictionary of Natural products, 2008). Also reported here is a derivative of flavoglaucin 3''',4'''-dehydroflavoglaucin **(8)** and 5''',6'''-dehydroflavoglaucin **(8a)** which were in a mixture.

4.5 ANTIPLASMODIAL ACTIVITIES

The crude CH_2Cl_2/MeOH (1:1) extract of the roots of *Kniphofia thomsonii* showed antiplasmodial activity with an IC_{50} value of 6.36±0.195 µg/ml against the chloroquine-resistant (W2) strain of *Plasmodium falciparum*.

The isolated compounds from this extract were tested and activities were observed for a phenylanthraquinone, two dimeric anthraquinone as well as two benzaldehyde derivatives (Table 13). These compounds appear to be partly responsible for the antiplasmodial activity of the crude extract. The phenylanthraquinone derivative knipholone **(6)** has good antiplasmodial activity with an IC_{50} value of 2.50±0.100 µg/ml against *P. falciparum* (W2 strain) which is comparable (IC_{50} value of 1.49 µg/ml) with what has been reported for the same compound isolated from *Kniphofia foliosa* in literature (Wube *et al.*, 2005). This difference in antiplasmodial activity could be attributed to the difference in stereochemistry of this compound and that reported in literature. In addition, antiplasmodial activity against asexual erythrocytic stages of two strains of *Plasmodium falciparum in vitro* (K1/chloroquine-resistant and NF 54 / chloroquine-sensitive) has also been reported (Bringmann *et al.*, 1999).

The dimeric anthraquinone derivative 10-hydroxy-10-(islandicin-7'-yl)-chrysophanol anthrone **(12)** showed good activity against the chloroquine resistant (W2) strain of *P. falciparum* with an IC_{50} value of 3.42±0.110 µg/ml whereas a strong antiplasmodial activity against the chloroquine sensitive 3D7 strain of *P. falciparum* with an IC_{50} value of 0.537 µg/mL has also been reported for the same compound (Wube *et al.*, 2005). The difference in the strains used and the stereochemistry of the compound influences the

antiplasmodial activities and thus the disparities observed. The other dimeric anthraquinone derivative 10,10'-bichrysophanol anthrone (**9**) showed good antiplasmodial activity against the chloroquine resistant (W2) strain of *P. falciparum* with an IC_{50} value of 2.23±0.022 µg/ml. This is the first report on the antiplasmodial activity of compound **9**.

The benzaldehyde derivatives on the other hand, showed good antiplasmodial activities against the chloroquine resistant (W2) strain of *P. falciparum* with compound **7** showing an IC_{50} value of 2.06±0.276 µg/ml whereas its derivative 3''',4'''-dehydroflavoglaucin (**8**) showed a better activity with an IC_{50} value of 1.93±0.784 µg/ml. An antifungal (mycotoxin) activity has been reported for this compound (Dictionary of Natural products, 2008), however this is the first report on its antiplasmodial activity.

This investigation has showed the potential of phenylanthraquinones, dimeric anthraquinone (with an anthrone and anthraquinone dimer structure) and the benzaldehyde derivatives as lead structures for development of antimalarial drugs which could be clinically useful to combat the malaria menace.

Table 13: *In vitro* IC_{50} values against the chloroquine resistant (W2) strain of *P. falciparum*

Sample	IC_{50} µg/ml
Root extract of *Kniphofia thomsonii*	6.36±0.195
Knipholone (**6**)	2.50±0.100
Flavoglaucin (**7**)	2.06±0.276
3''',4'''-Dehydroflavoglaucin (**8**)	1.93±0.784
10,10'-bichrysophanol anthrone (**9**)	2.23±0.022
10-hydroxy-10-(islandicin-7'-yl)-chrysophanol anthrone (**12**)	3.42±0.110

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

- The crude CH_2Cl_2/MeOH (1:1) extract of the roots of *Kniphofia thomsonii* showed good antiplasmodial activity against the chloroquine-resistant (W2) strain of *Plasmodium falciparum* and thus its use as an antimalarial agent traditionally is justified.

- From the roots of *Kniphofia thomsonii*, a total of thirteen compounds were isolated and characterized. These include the monomeric anthraquinones: chrysophanol (**1**), islandicin (2), physcion (3), aloe-emodin acetate (**4**) and aloe-emodin (**5**); the phenylanthraquinone: knipholone (**6**); the benzaldehyde derivatives: flavoglaucin (**7**) and 3''',4'''-dehydroflavoglaucin (**8**) and the dimeric anthraquinones: 10,10'-bichrysophanolanthrone (**9**), 10-hydroxy-10-(chrysophanol-7'-yl) chrysophanolanthrone (**10**), 10-hydroxy-10-(chrysophanol-7'-yl)-aloe-emodinanthrone (**11**), 10-hydroxy-10-(islandicin-7'-yl)-chrysophanolanthrone (**12**) and 10-hydroxy-10-(islandicin-7'-yl)-aloe-emodinanthrone (**13**). The dimeric anthraquinone **13** is a new compound while flavoglaucin (**7**) and 3''',4'''-dehydroflavoglucin (**8**) are reported for the first time in higher plants; the C-6 oxygenated anthraquinone physcion (3) is reported for the first time in the family Asphodelaceae; and this is also the first report for the occurrence of compound **9** (10,10'-bichrysophanolanthrone) in the genus *Kniphofia*.

- The anti-plasmodial activity of the crude CH_2Cl_2/MeOH (1:1) extract of *Kniphofia thomsonii* could be attributed to the phenylanthraquinone, dimeric anthraquinones and the benzaldehyde derivatives with the highest activity being attributed to the phenylanthraquinone **6** and the benzaldehyde derivative **8**. Prior to this report, not much has been reported on the antiplasmodial activities of dimeric anthraquinones.

All the dimeric anthraquinones isolated and tested in this study showed good activities against (W2) strain

5.2 RECOMMENDATION

- It is recommended that the antiplasmodial activity of the compounds isolated in this study to be tested against other strains of *Plasmodium*.

- It is recommended that *in vivo* testing of the anthraquinones be carried out.

- The structure-activity study with a wide range of anthraquinones should be done to determine the structural requirement for anti-plasmodial activity.

- The interest on the anthraquinones of the family Asphodelaceae should not be limited to antiplasmodial activities. Screening for other biological activities especially on a broader spectrum of antiprotozoal activities including anti-leishmaniasis is recommended.

- Further phytochemical work on the genus *Kniphofia* could lead to the isolation and characterization of more novel compounds with biological activities.

- It is recommended that studies on the stereochemistry of the dimeric anthraquinones be done.

- It is also recommended that further studies be done to establish the relationship between stereochemistry and the antiplasmodial activity of the phenylanthraquinones as well as the dimeric anthraquinones.

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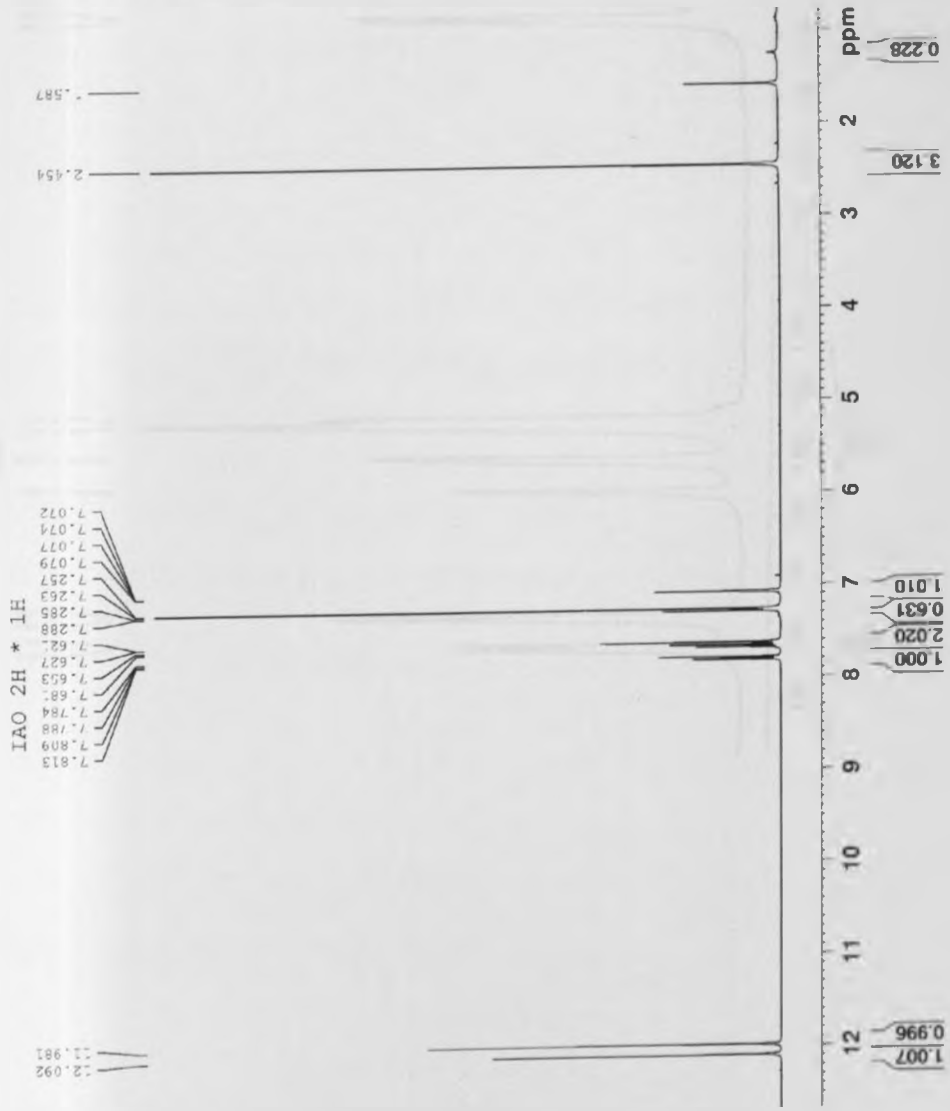
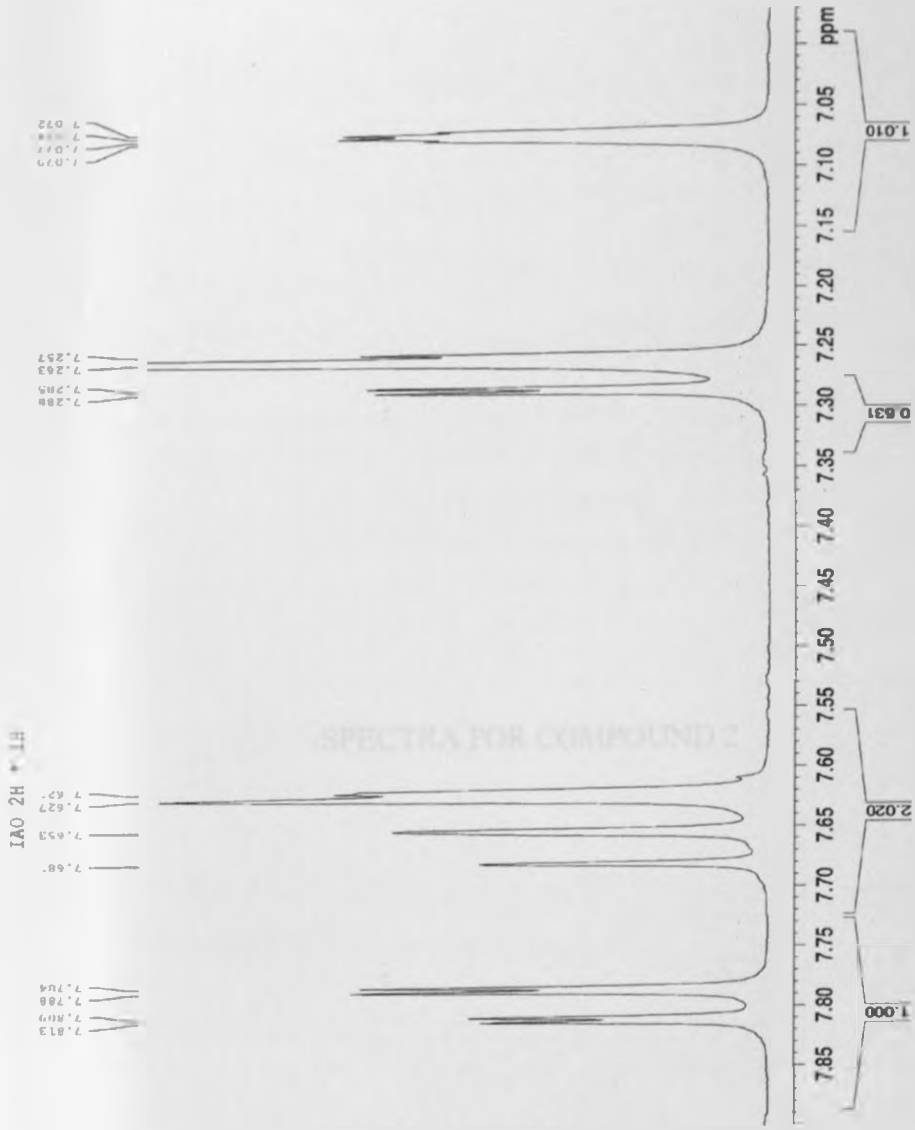
SPECTRA FOR COMPOUND 1

^{1}H NMR SPECTRUM FOR COMPOUND 1 (SOLVENT: $CDCl_3$, 300 MHz)



^{1}H NMR SPECTRUM FOR COMPOUND 1 (SOLVENT: $CDCl_3$, 300 MHz)



SPECTRA FOR COMPOUND 2

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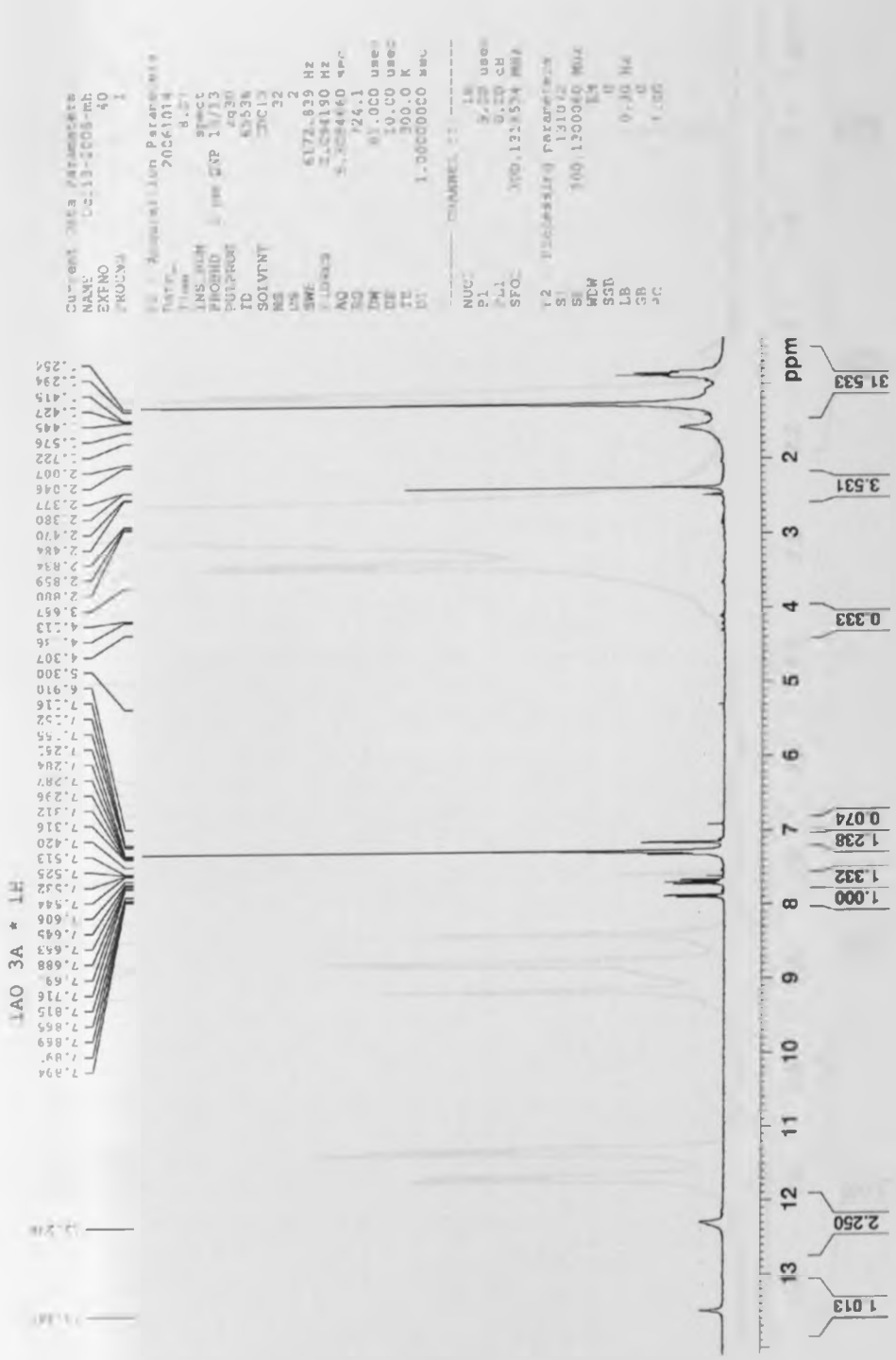

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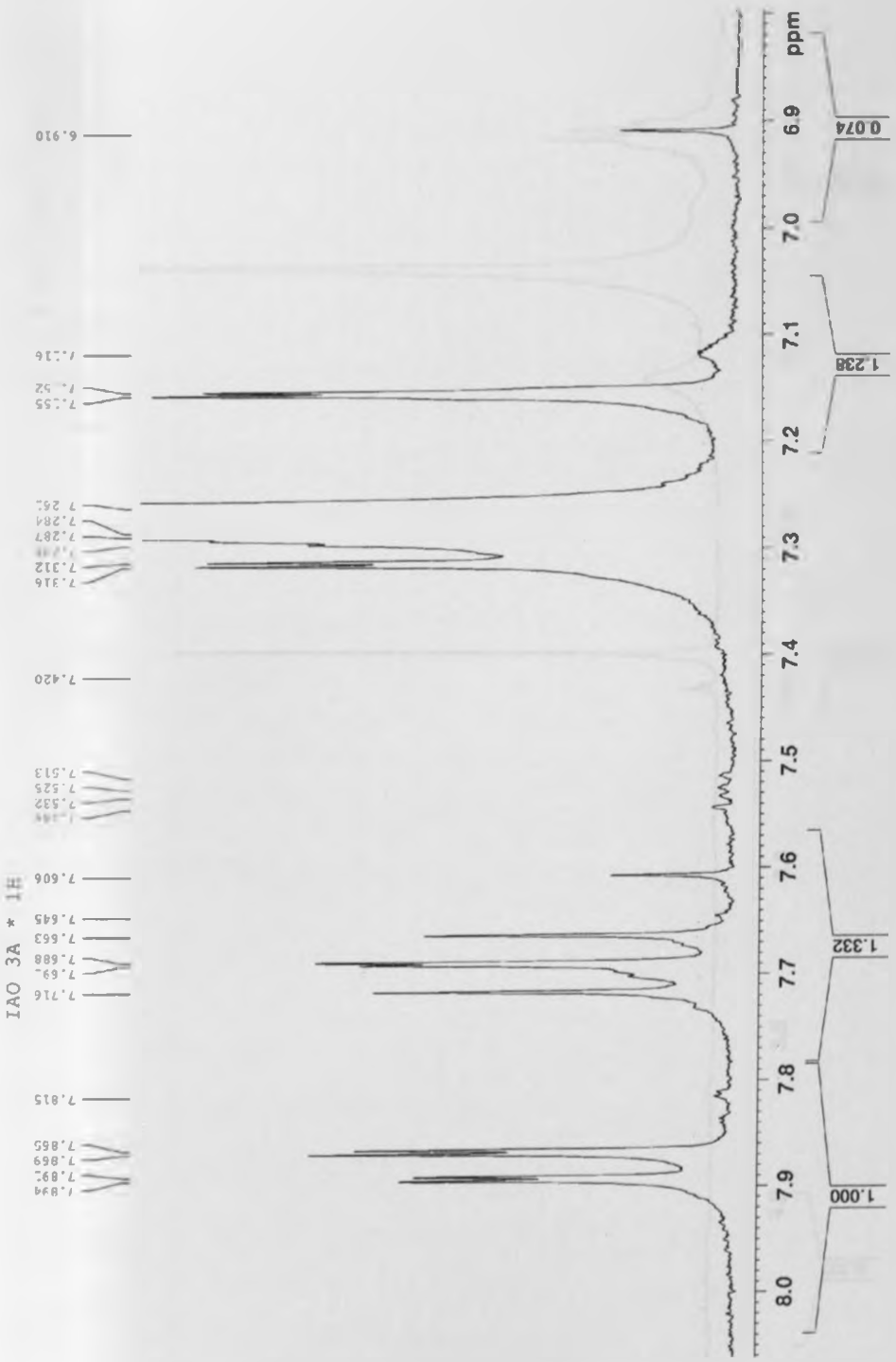
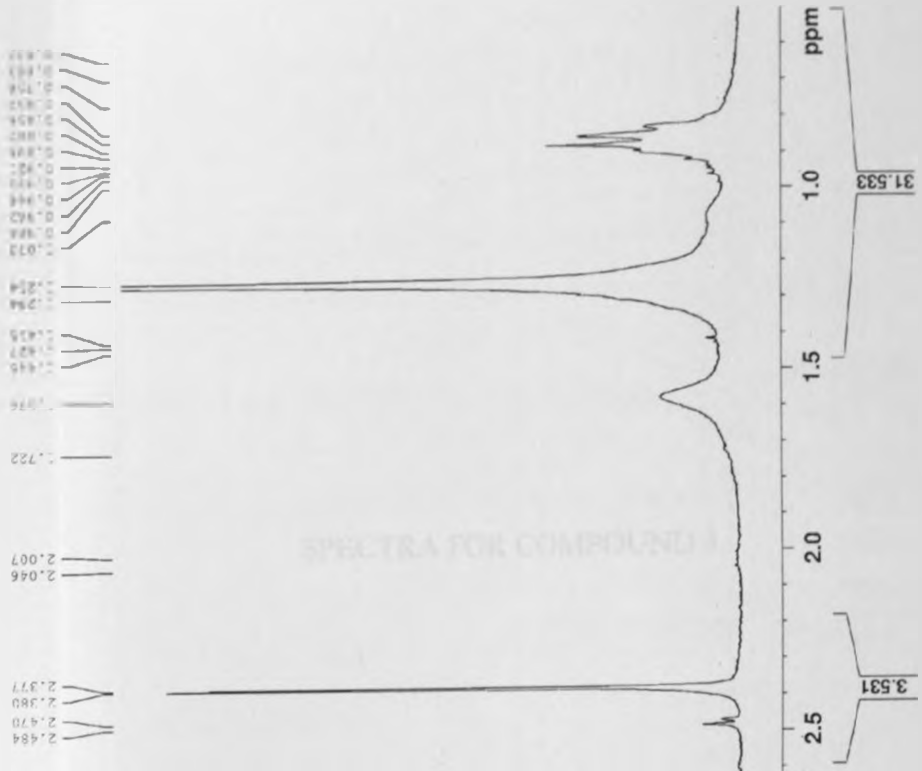


IAO 3A * 1H

4.307
4.336
4.313
3.667
2.880
2.859
2.834

4.0
3.5
3.0
0.333

^{1}H NMR SPECTRUM FOR COMPOUND 2 (SOLVENT: $CDCl_3$, 300 MHz)



SPECTRA FOR COMPOUND 3

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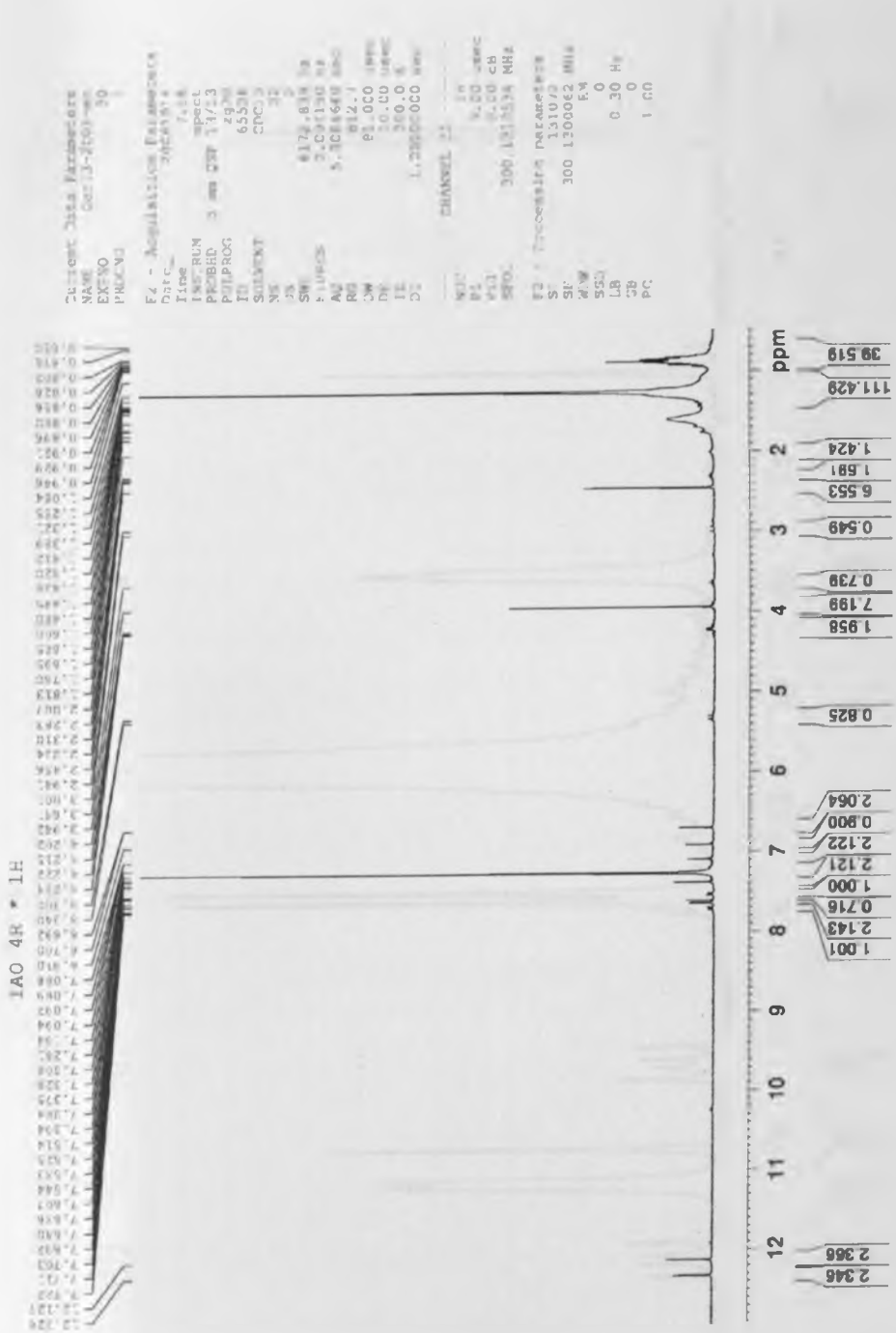

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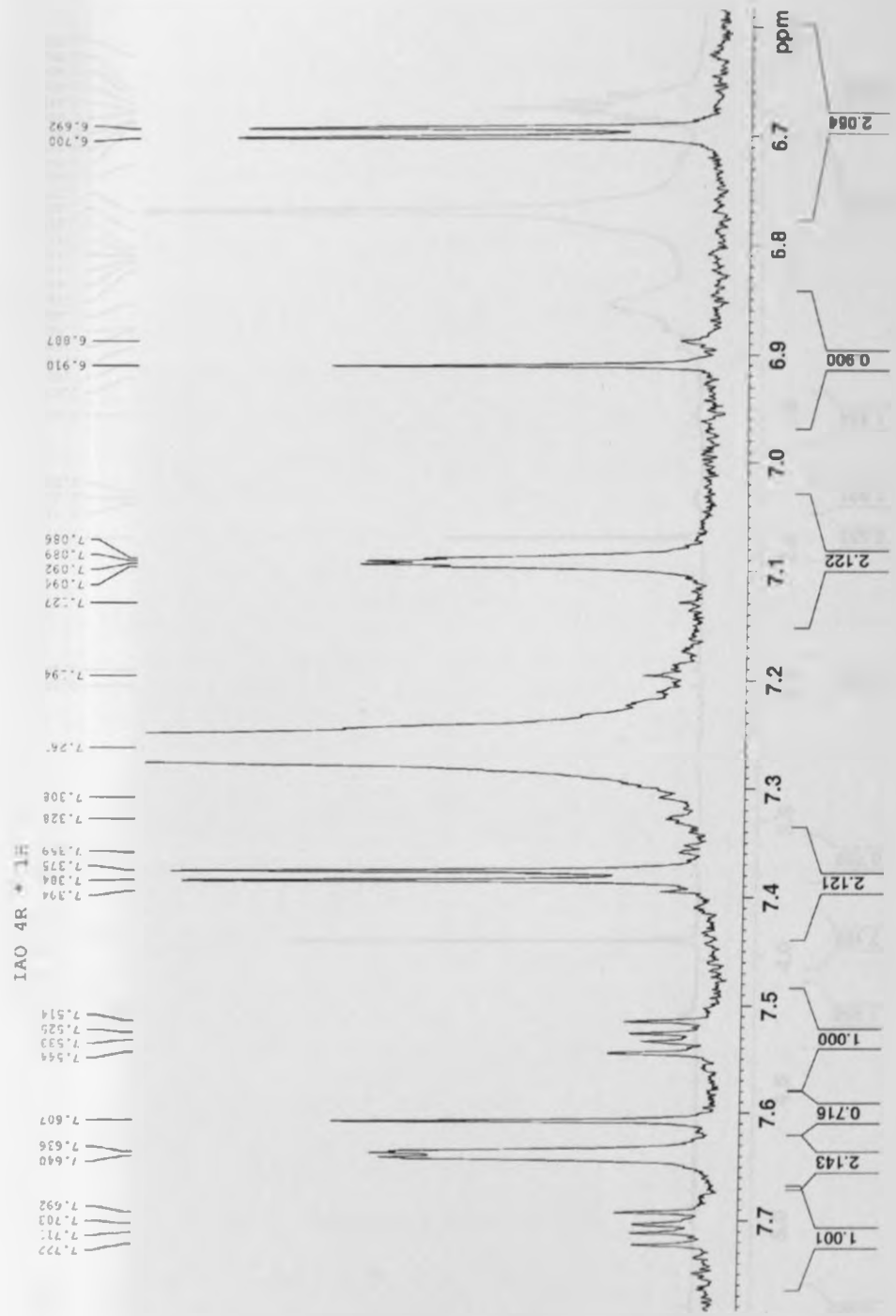
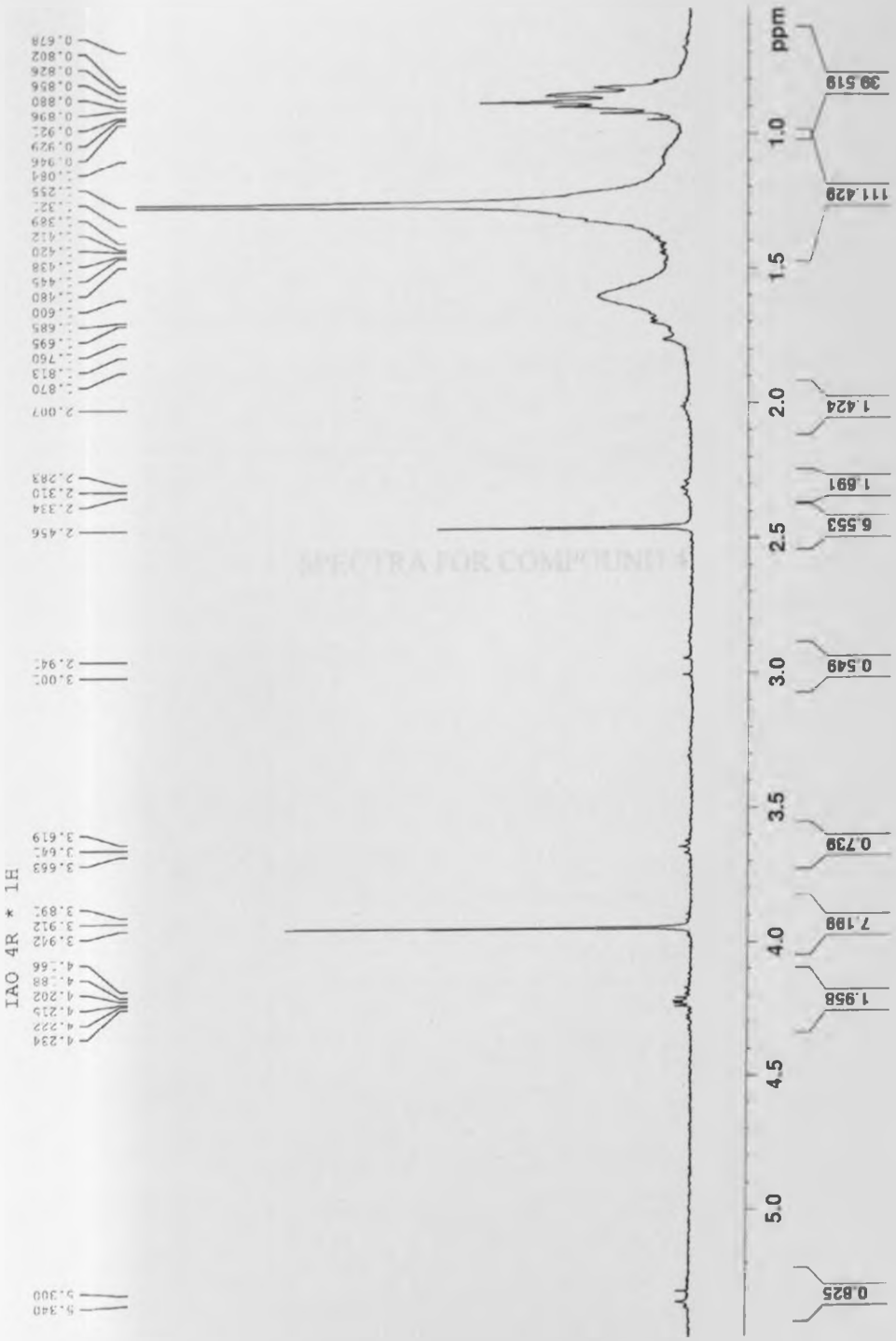
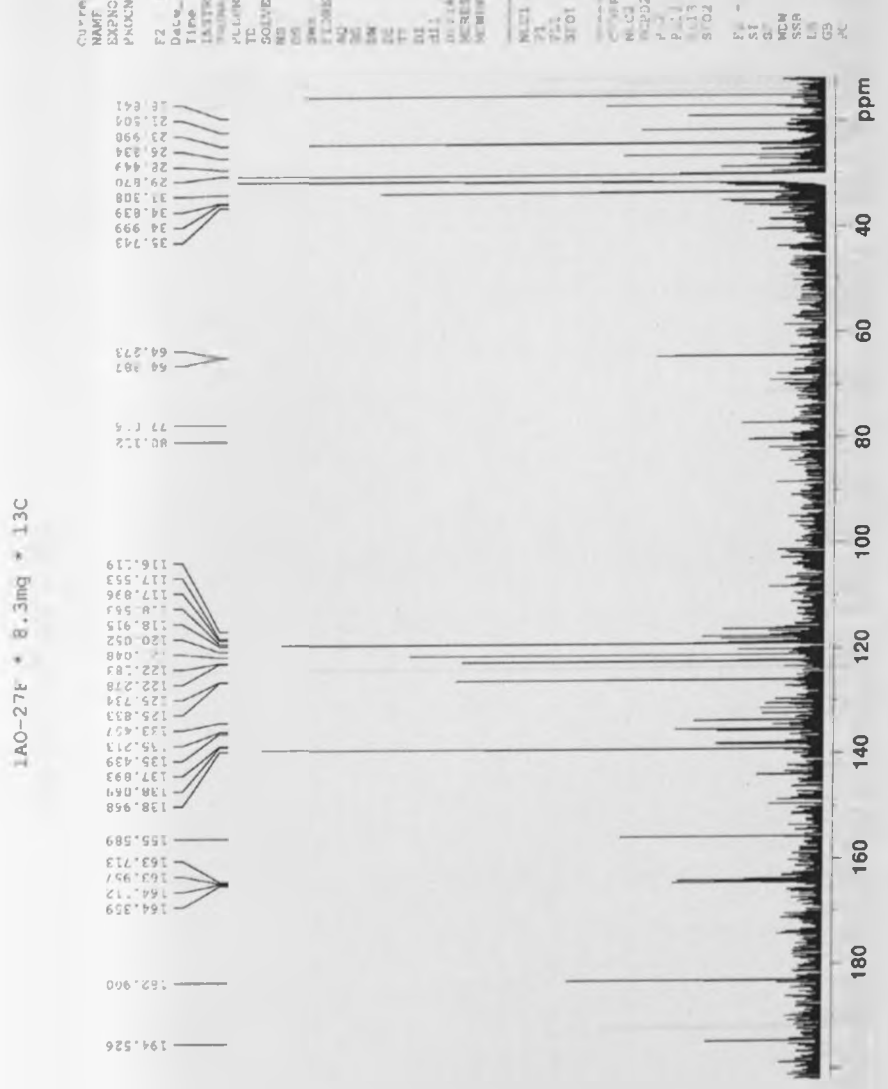


^{1}H NMR SPECTRUM FOR COMPOUND 3 (SOLVENT: $CDCl_3$, 300 MHz)

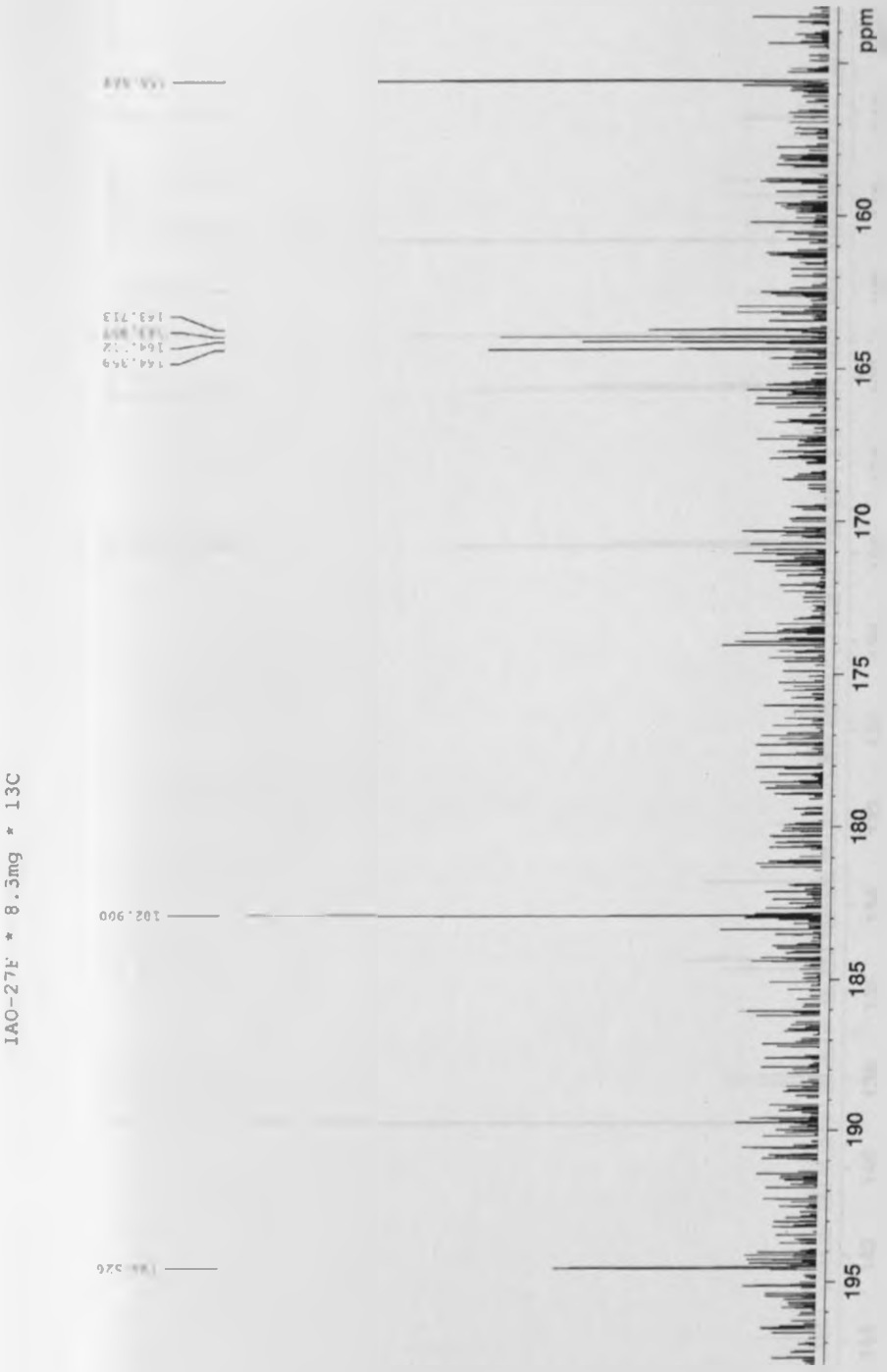


SPECTRA FOR COMPOUND 4

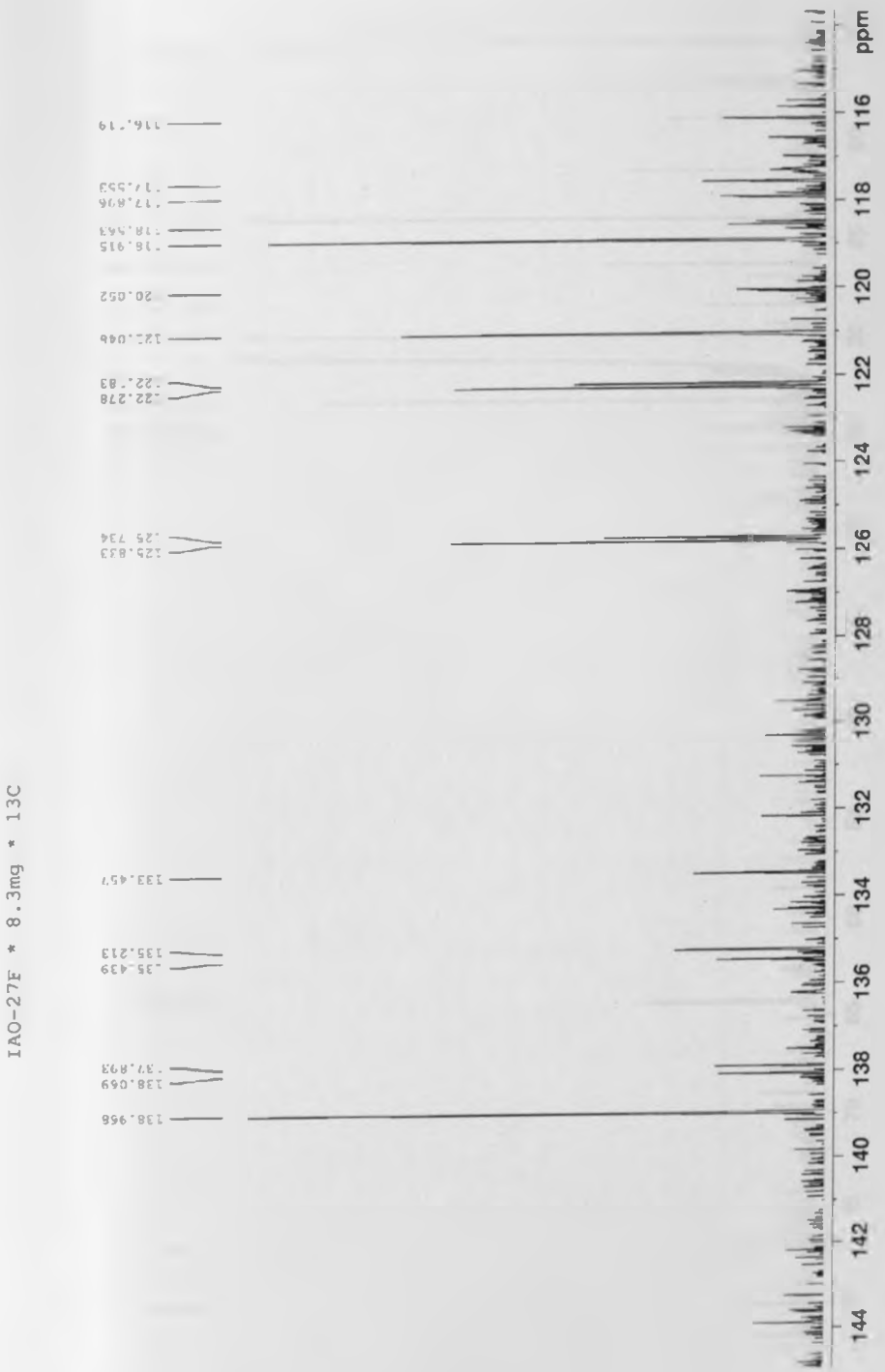
^{13}C NMR SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, 125MHz)



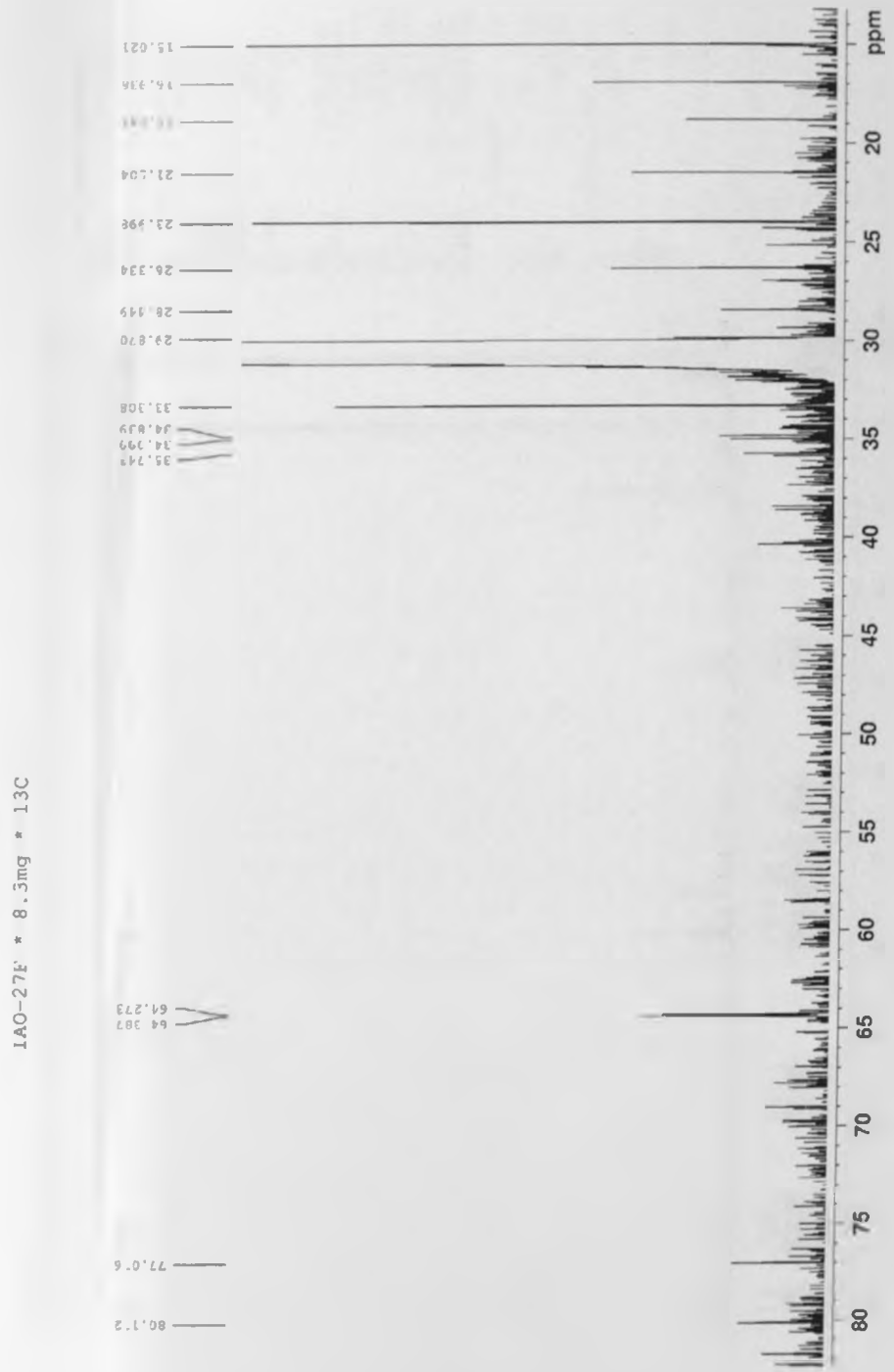
^{13}C NMR SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, 125MHz)



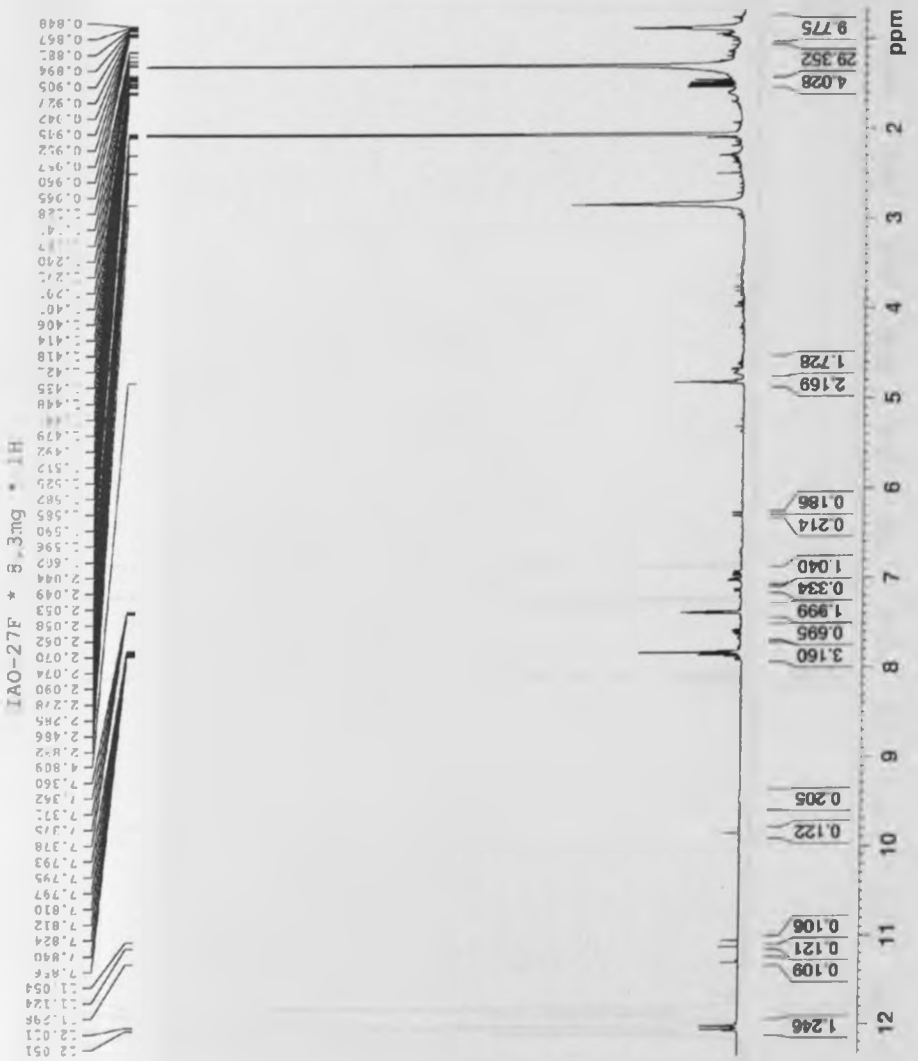
^{13}C NMR SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, 125MHz)



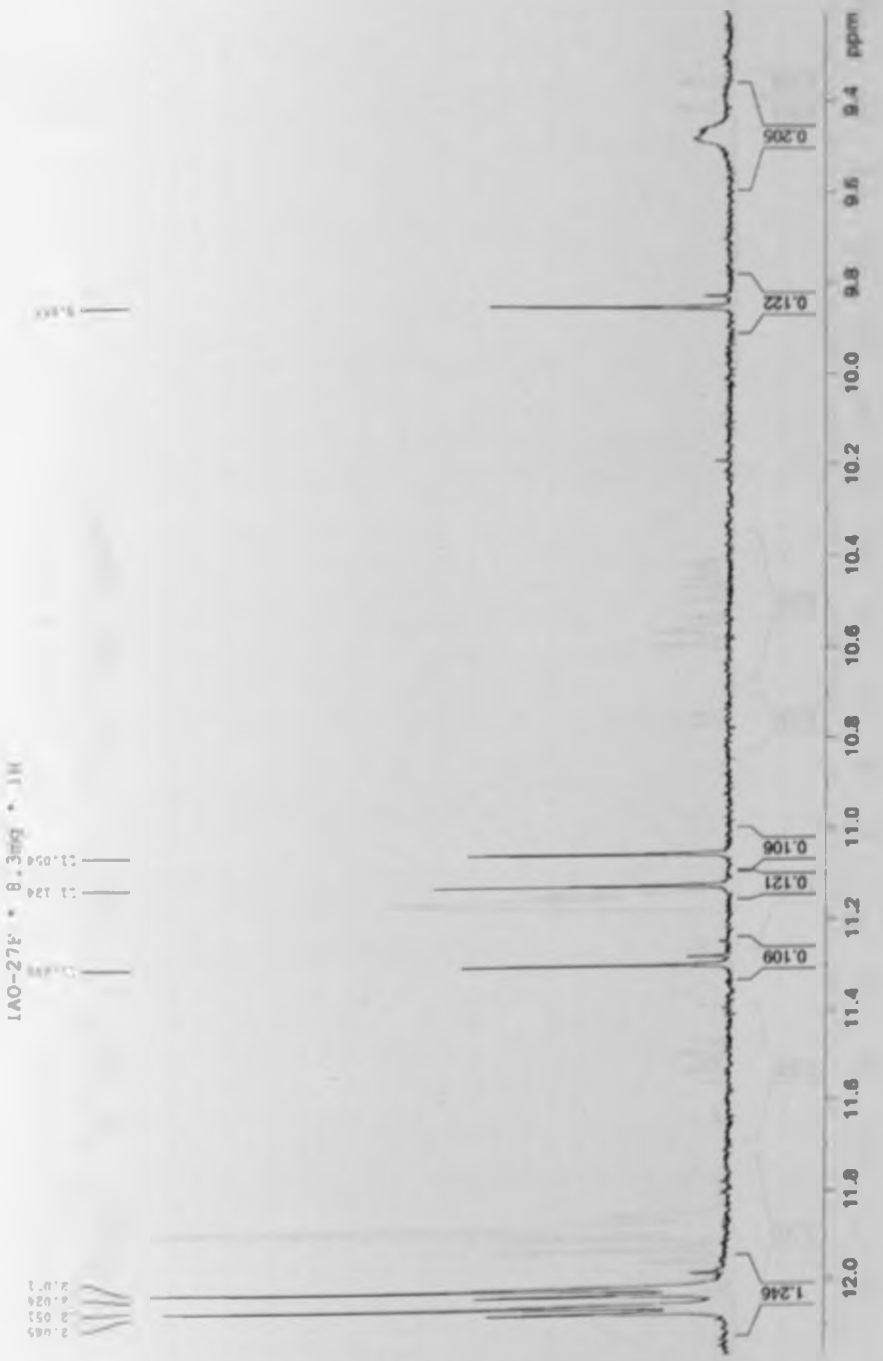
^{13}C NMR SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, 125MHz)



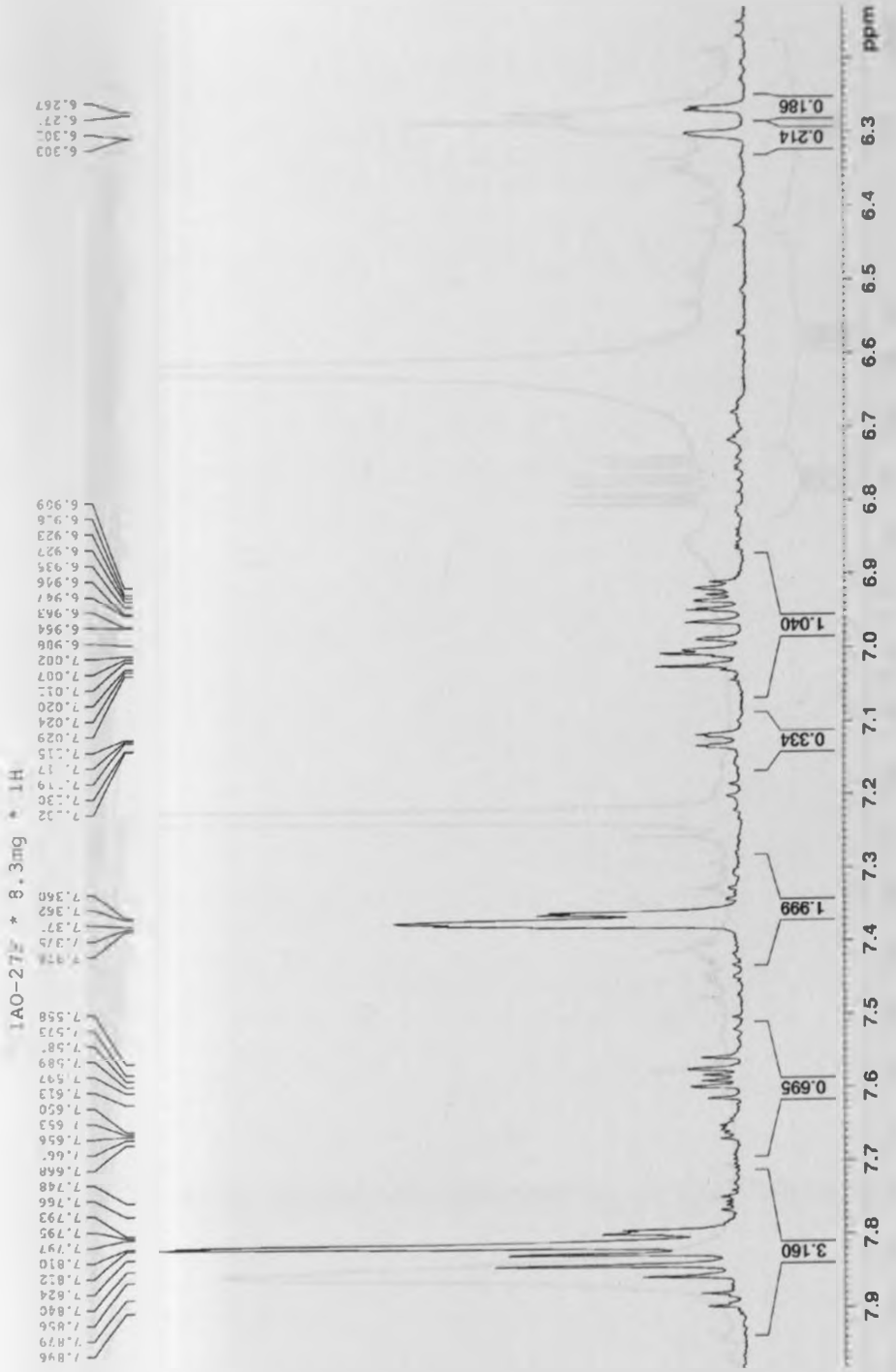
^{1}H NMR SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, 500 MHz)



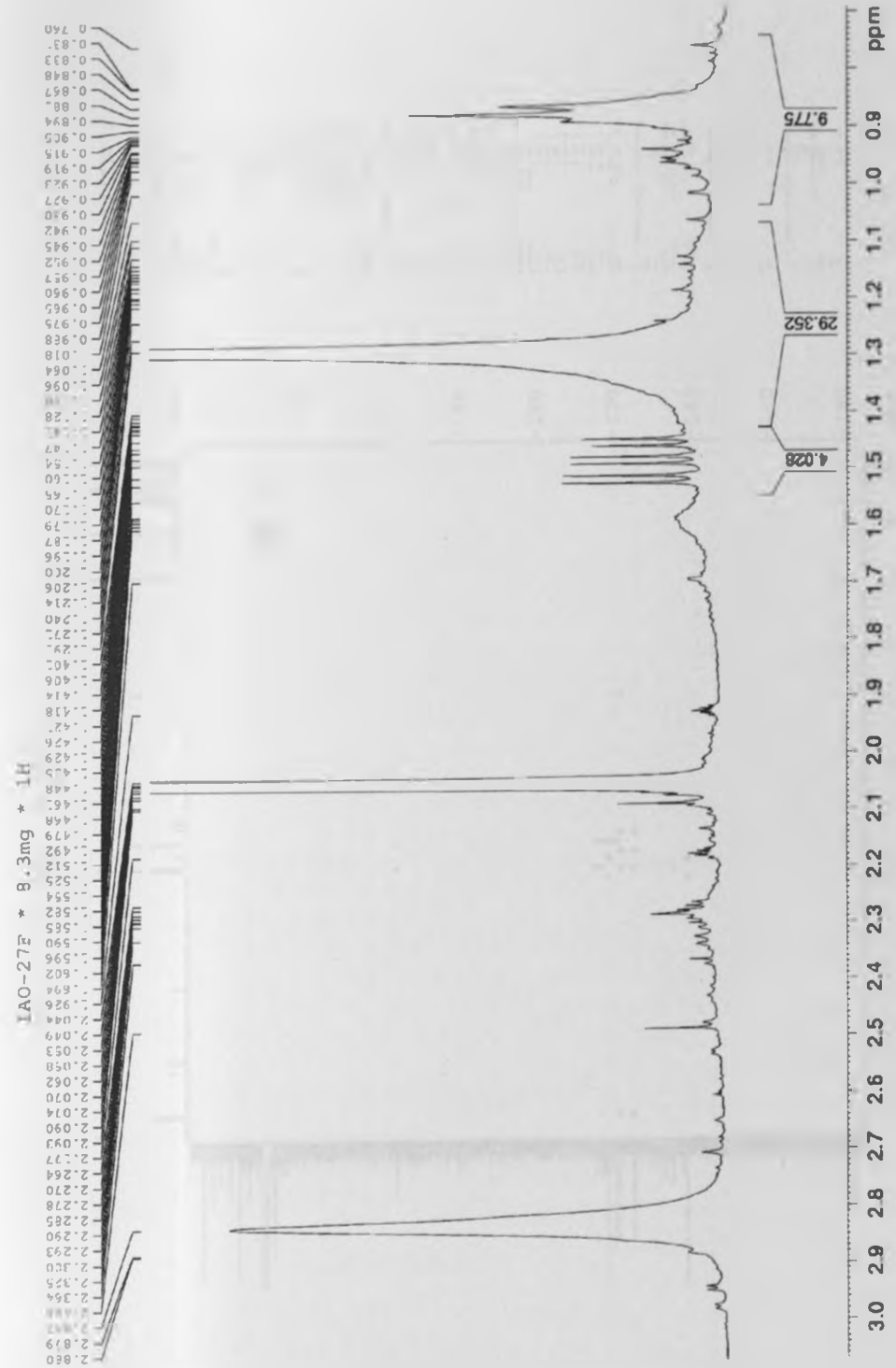
^{1}H NMR SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, 500 MHz)



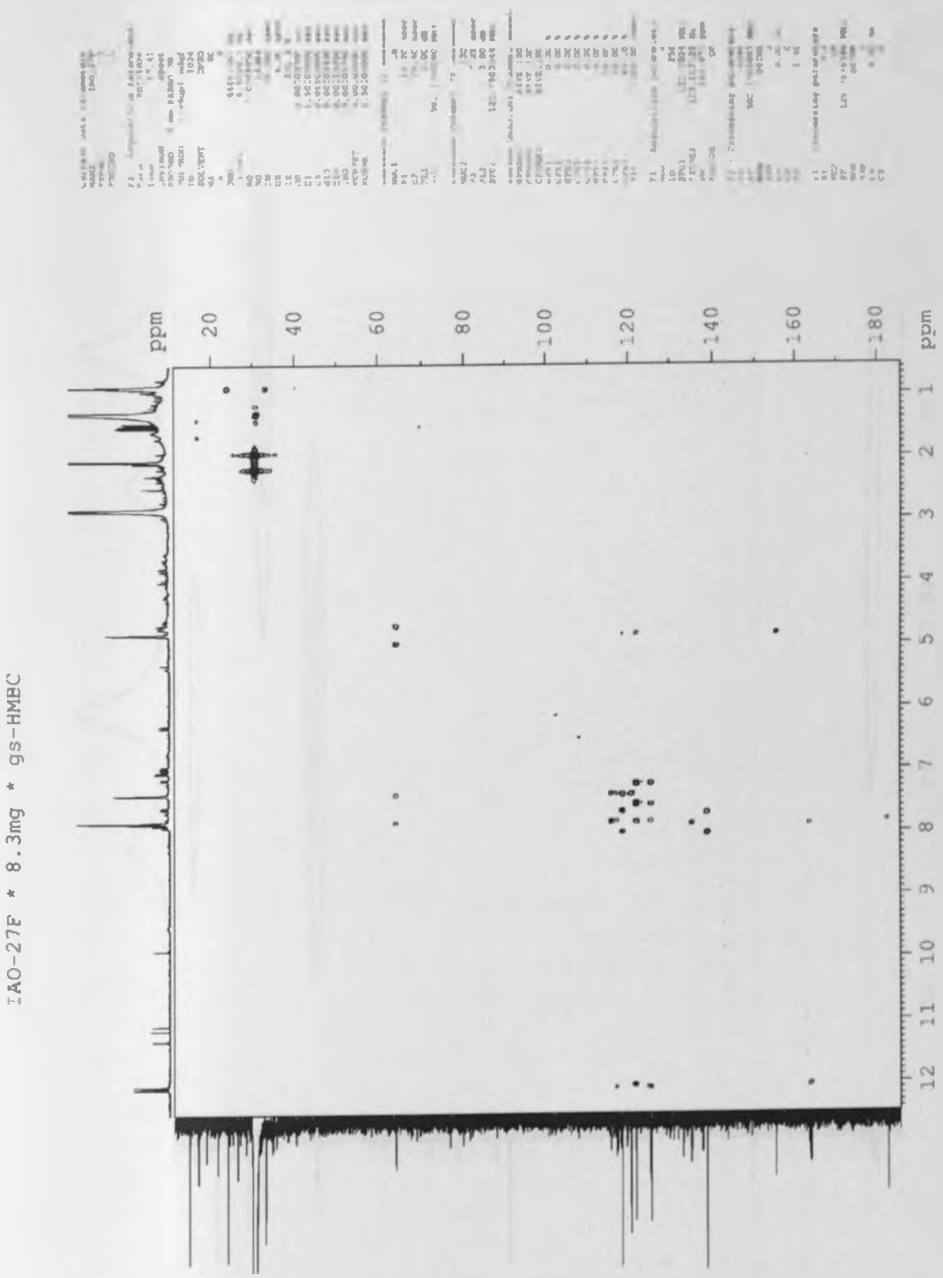
^{1}H NMR SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, 500 MHz)



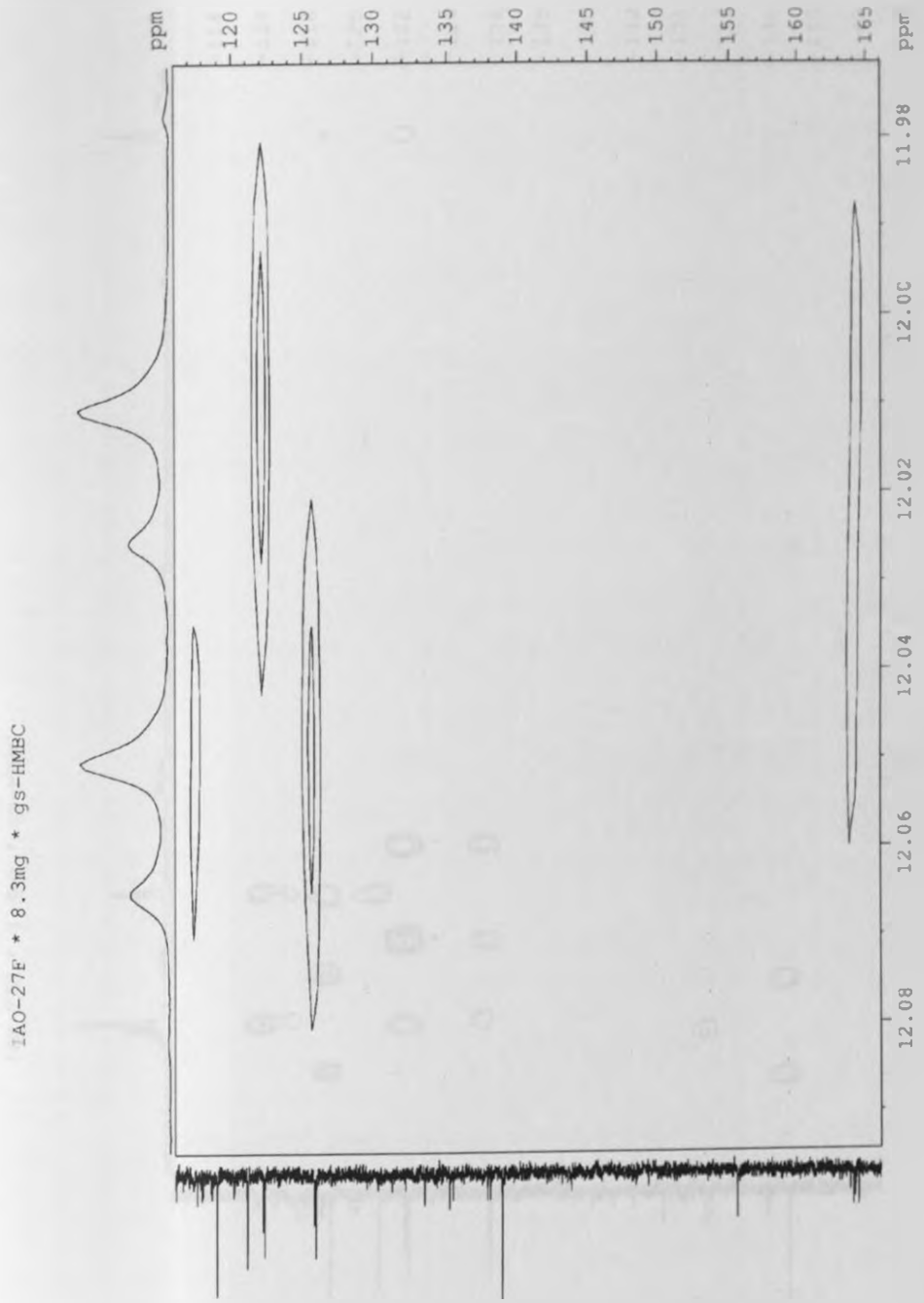
^{1}H NMR SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, 500 MHz)



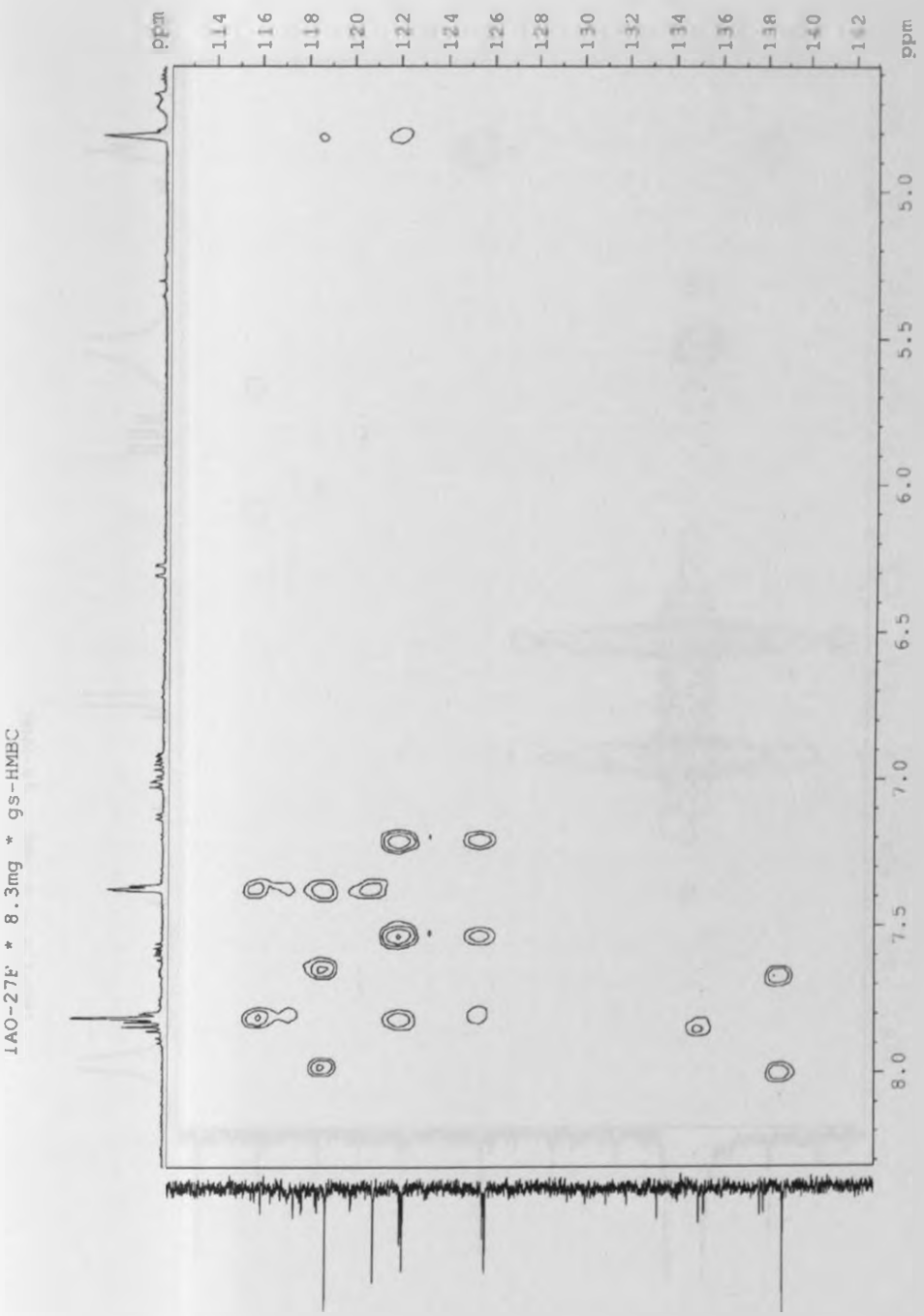
HMBC SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)



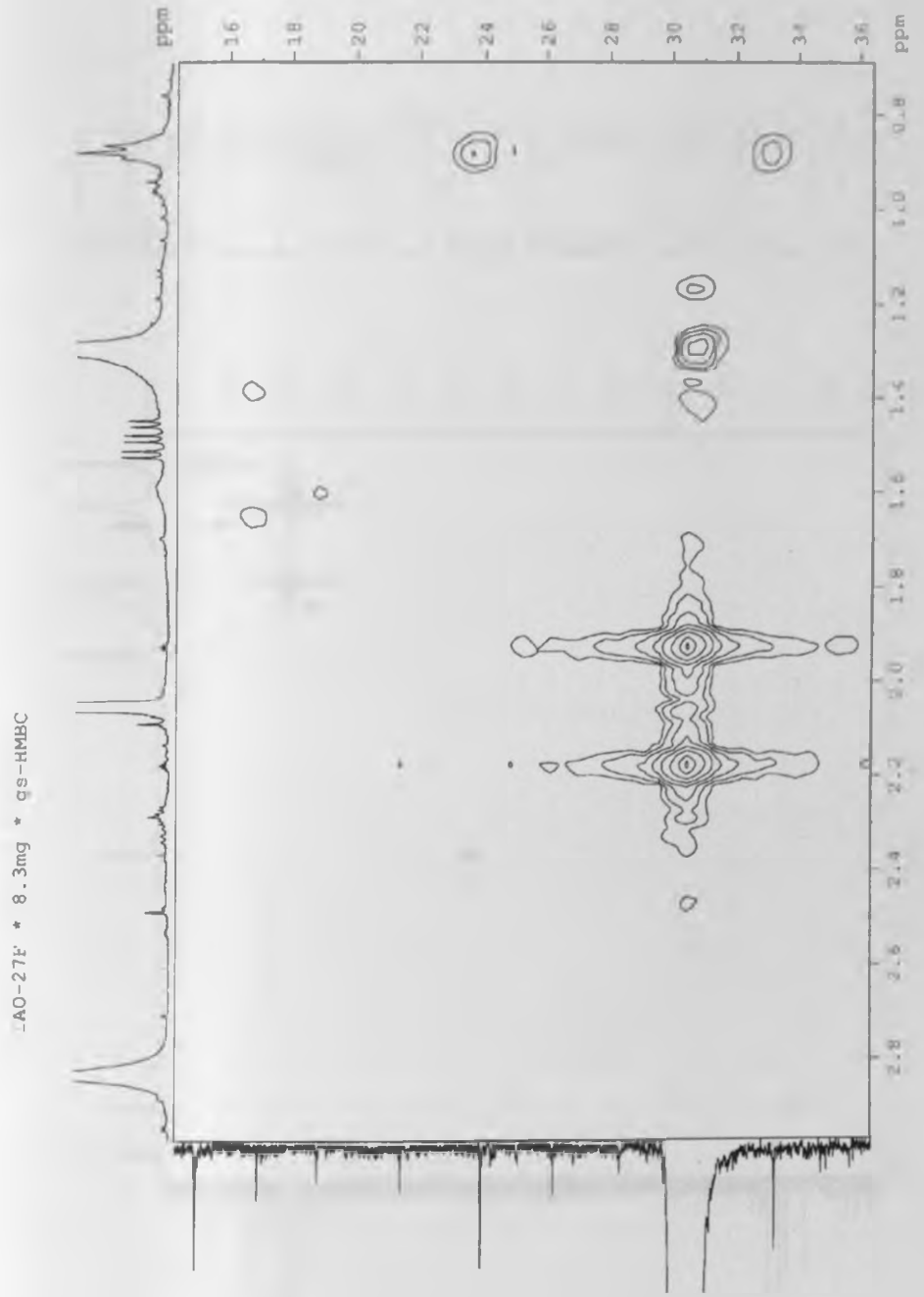
HMBC SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)



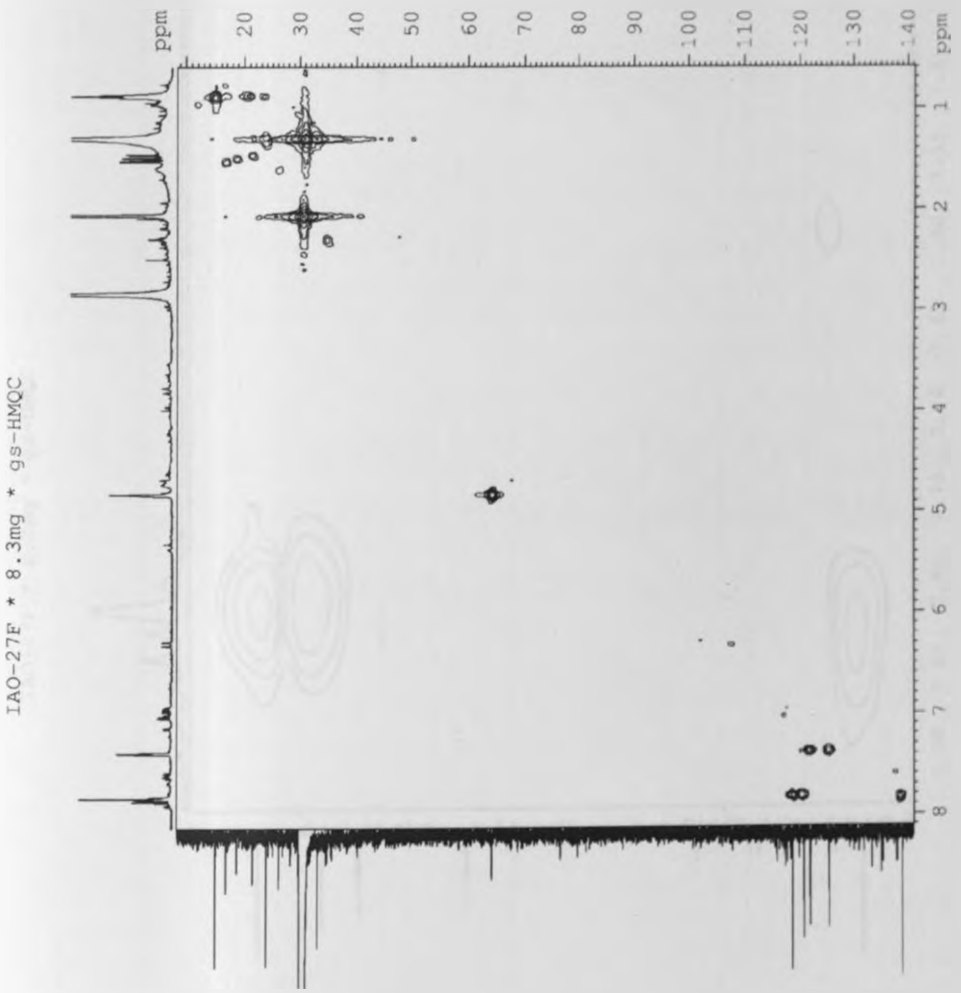
HMBC SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)



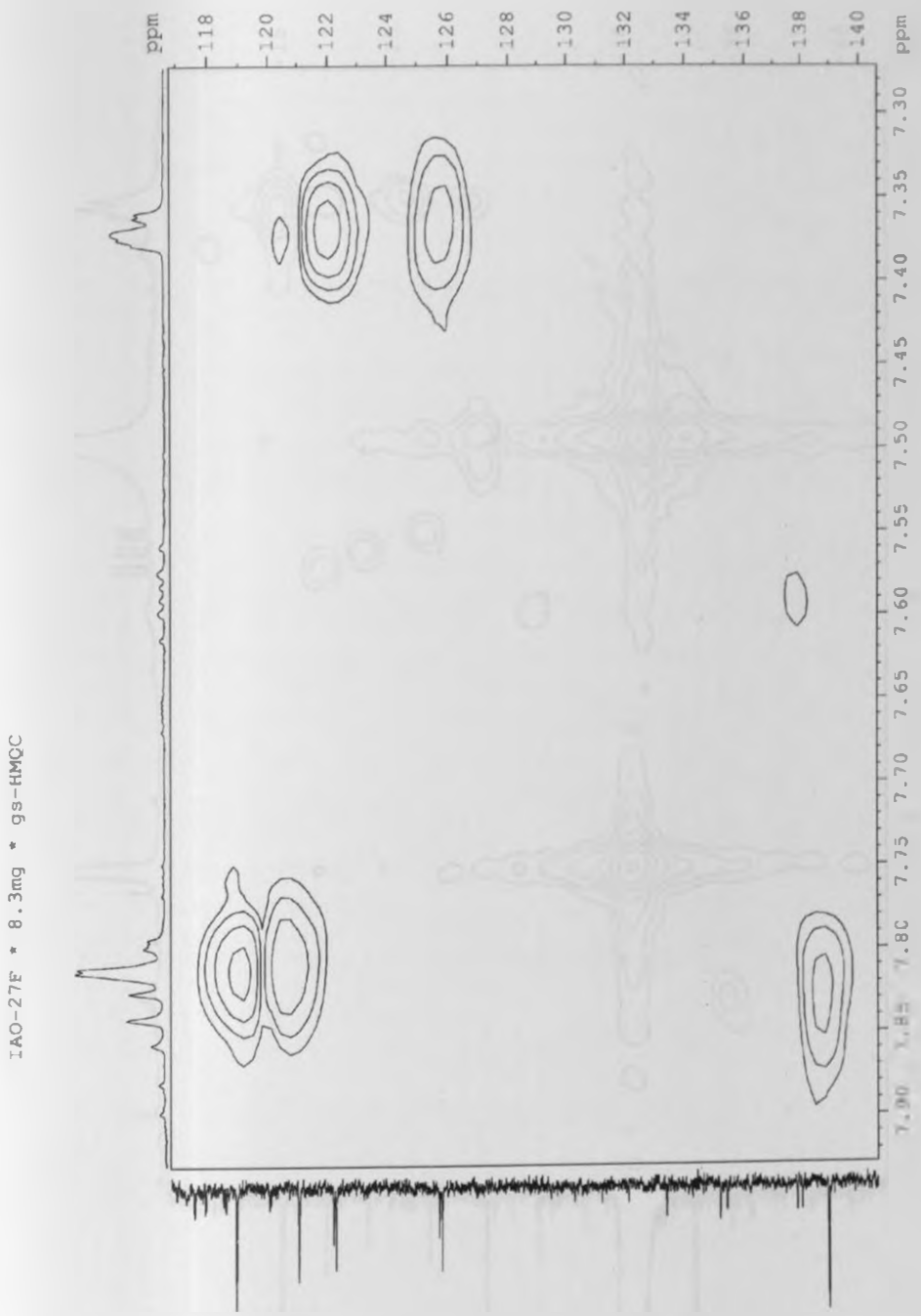
HMBC SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)



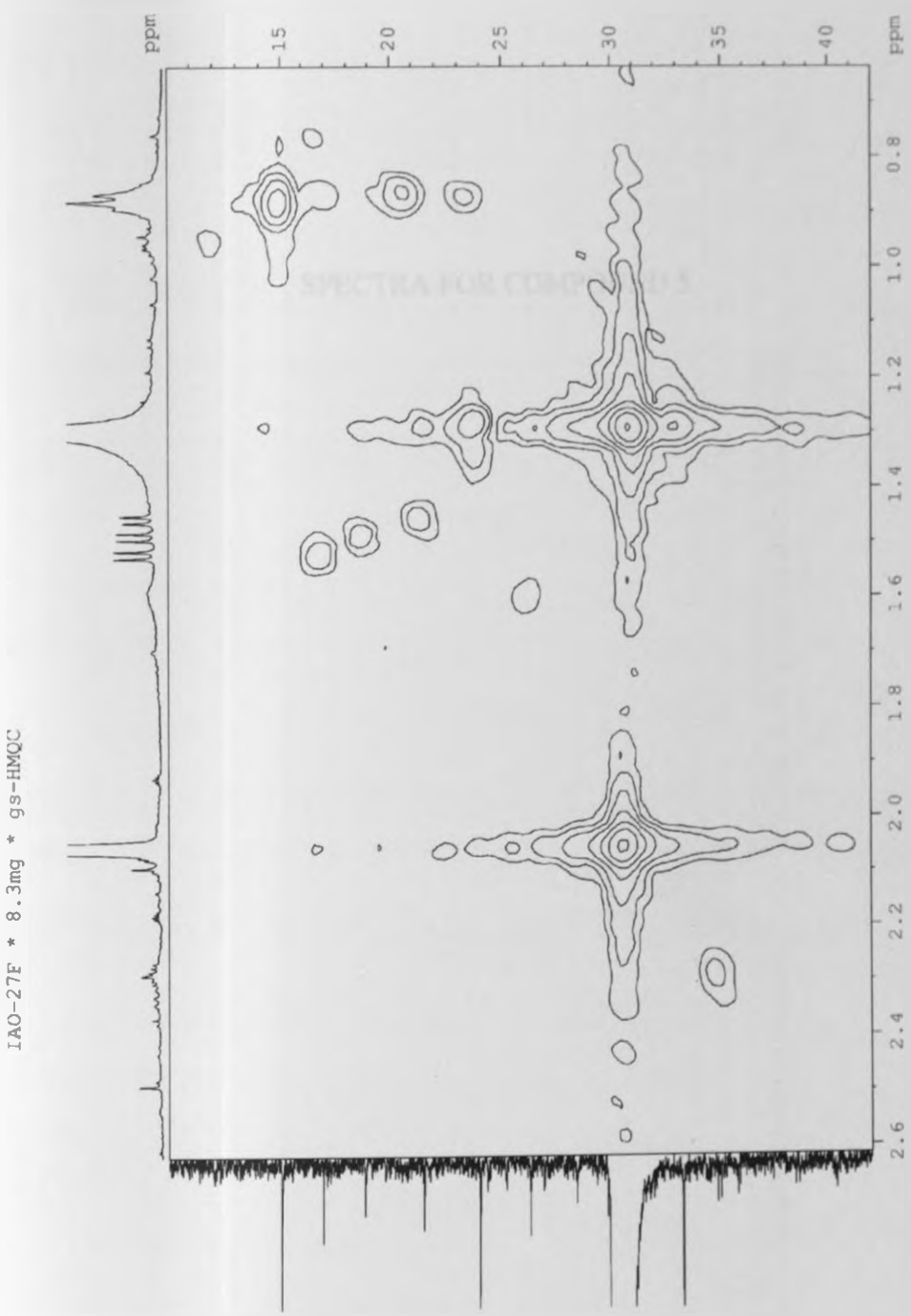
HMQC SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)



HMQC SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)

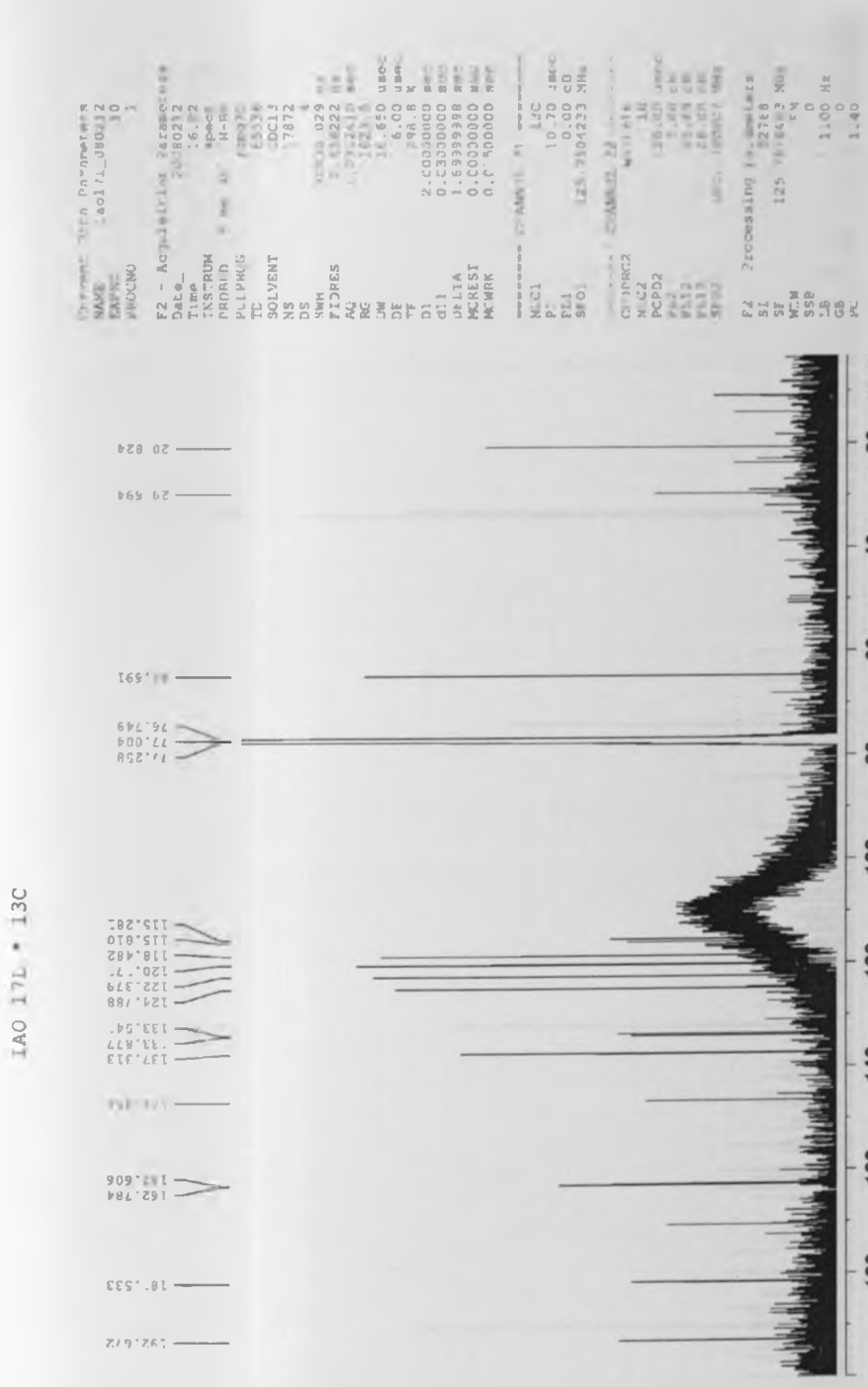


HMQC SPECTRUM FOR COMPOUND 4 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)



SPECTRA FOR COMPOUND 5

^{13}C NMR SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, 125MHz)



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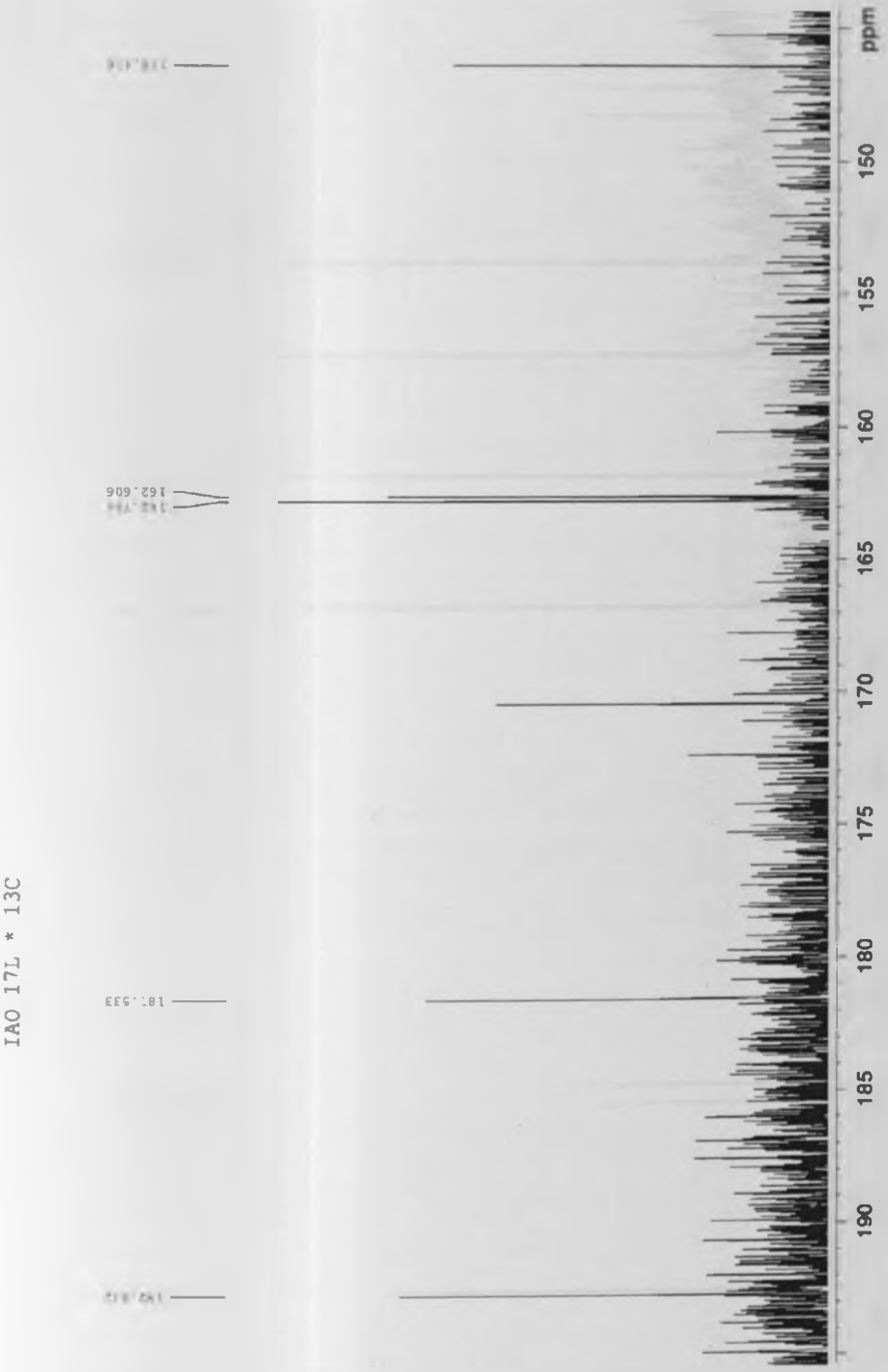
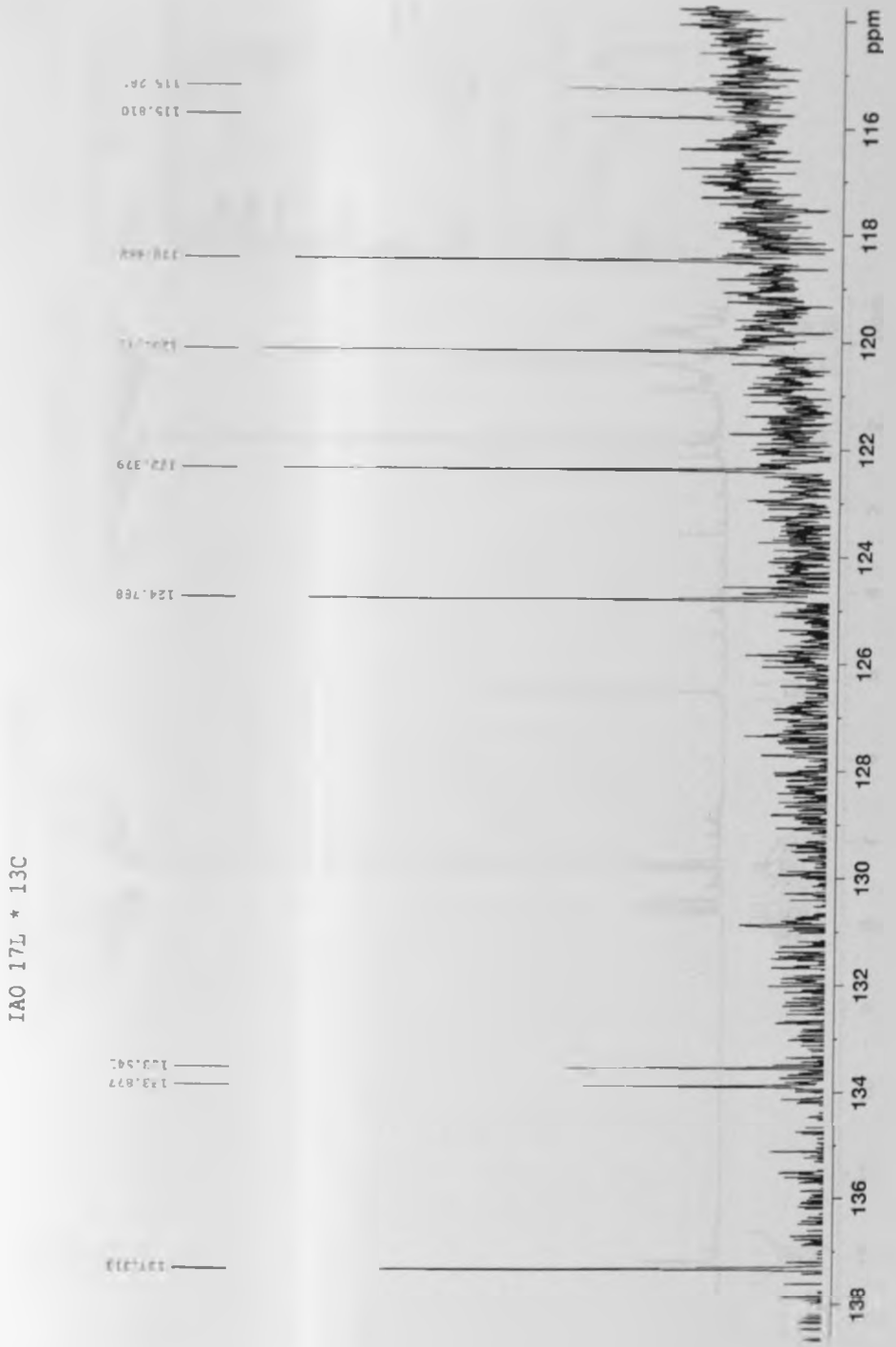
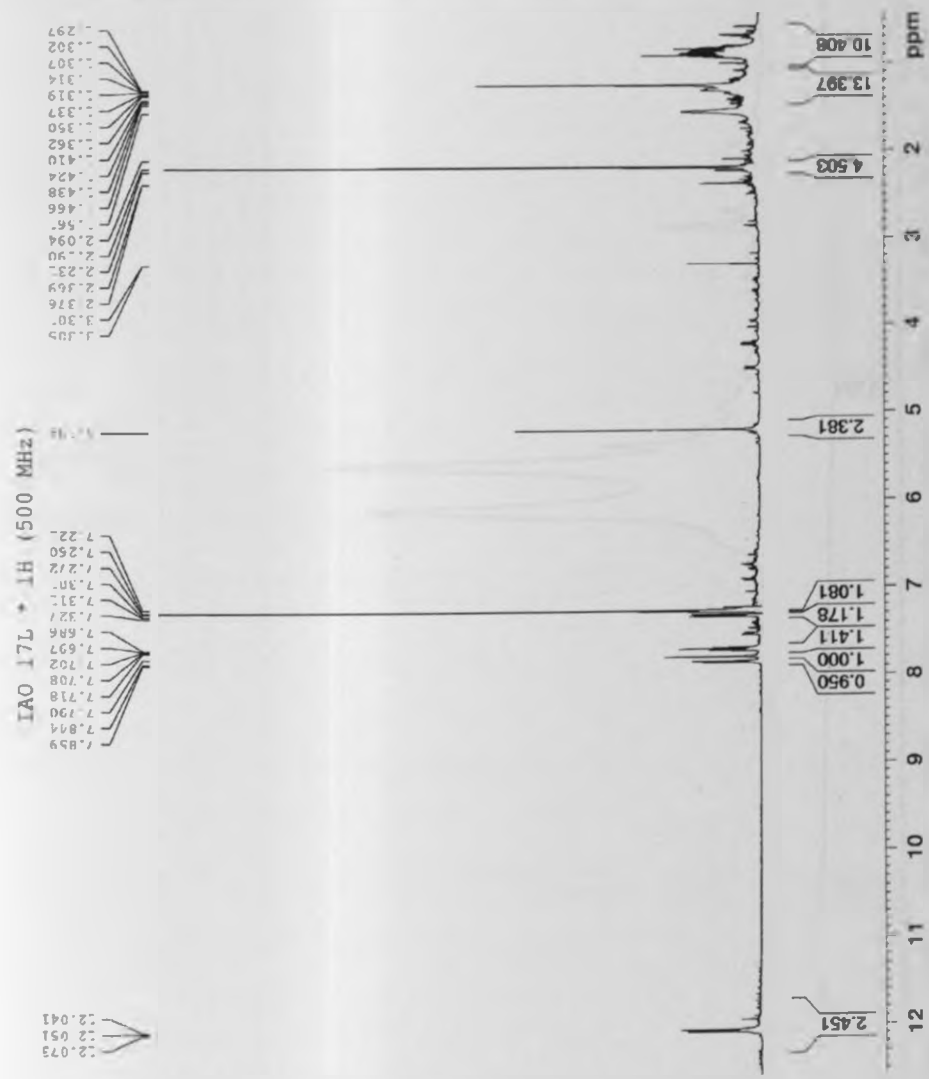


^{13}C NMR SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, 125MHz)

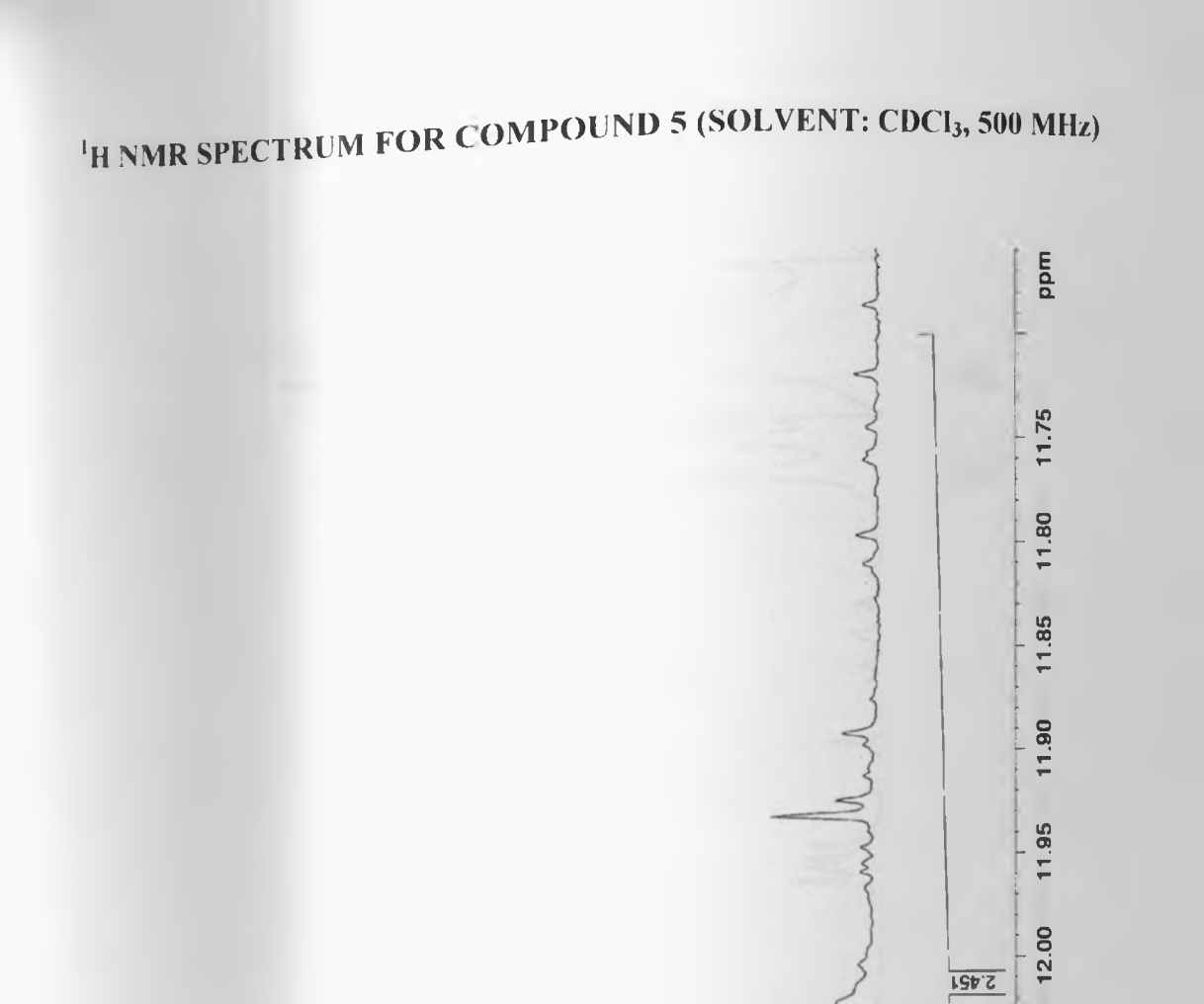


^{1}H NMR SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, 500 MHz)

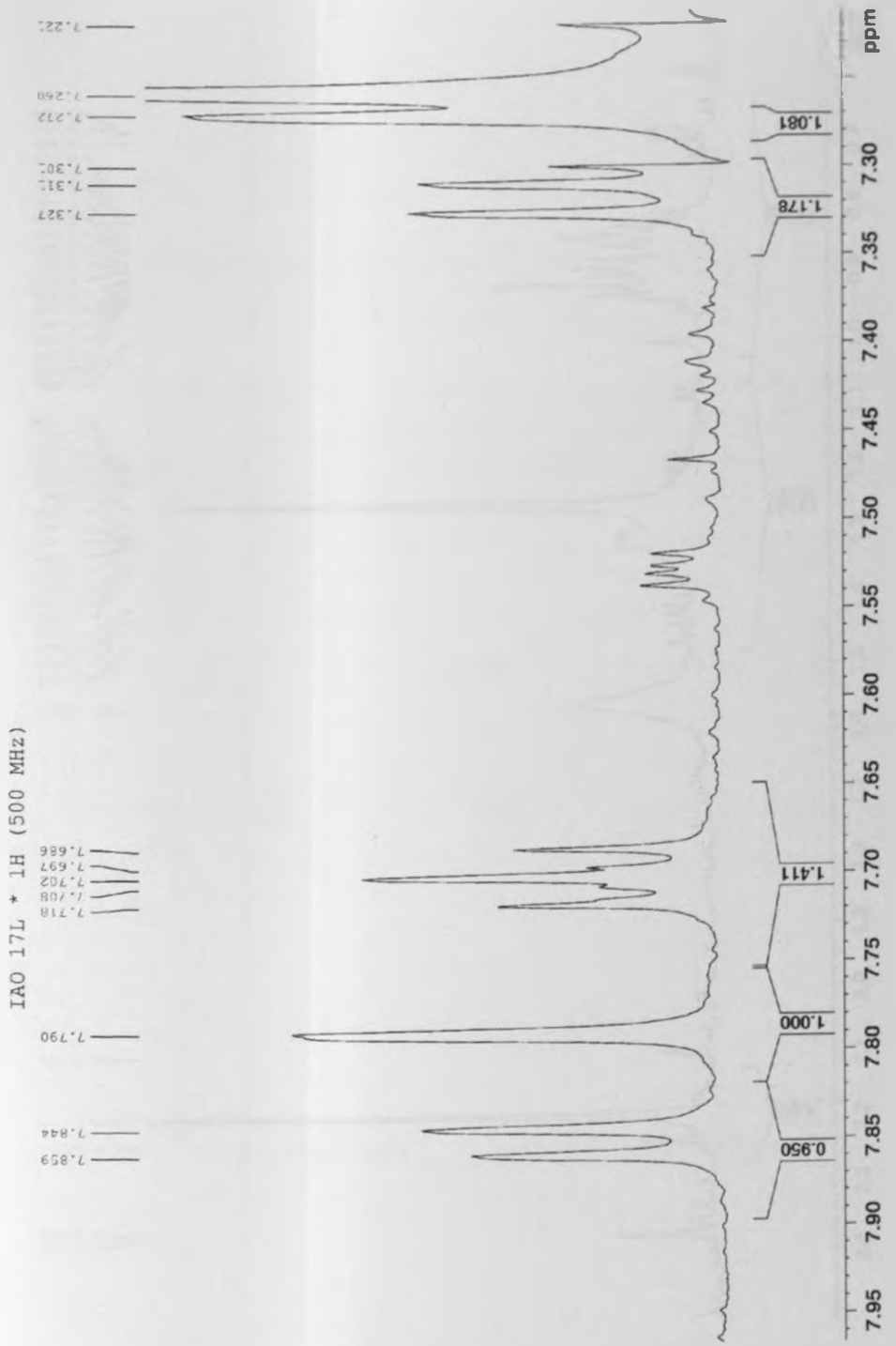


IAO 17L * 1H (500 MHz)
12.073
12.051
12.041
12.35
12.30
12.25
12.20
12.15
12.10
12.05

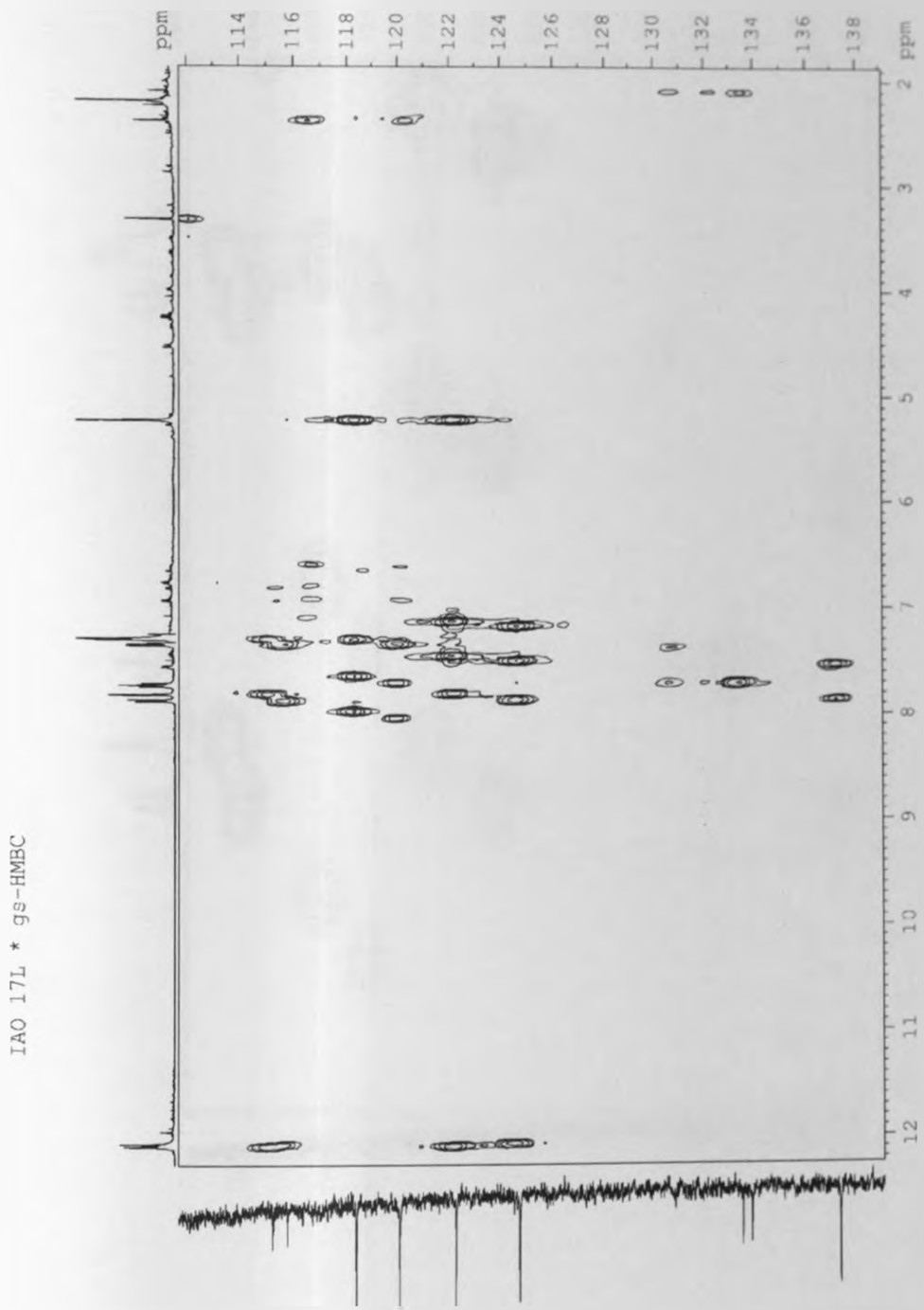
^{1}H NMR SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, 500 MHz)



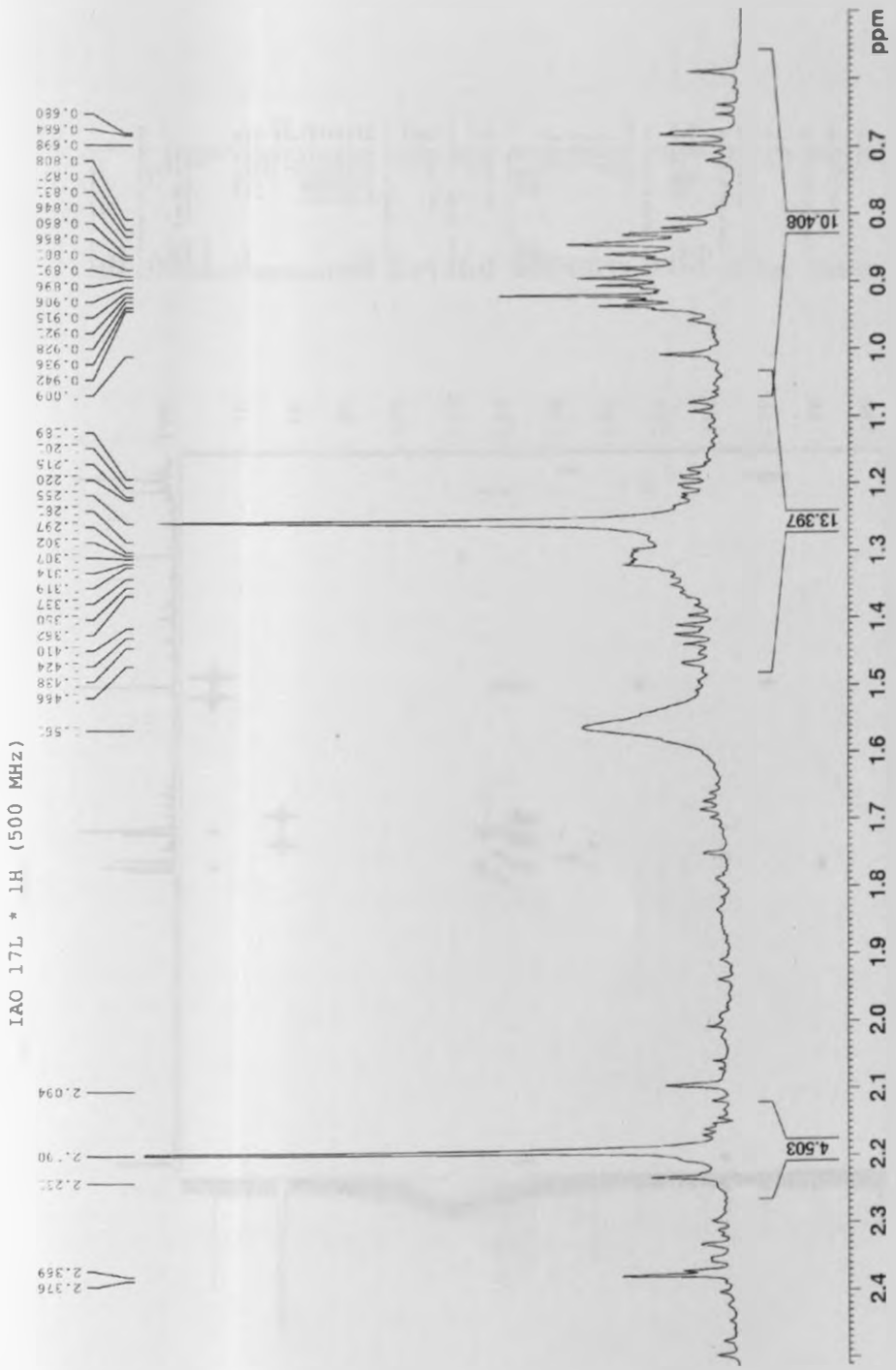
^{1}H NMR SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, 500 MHz)



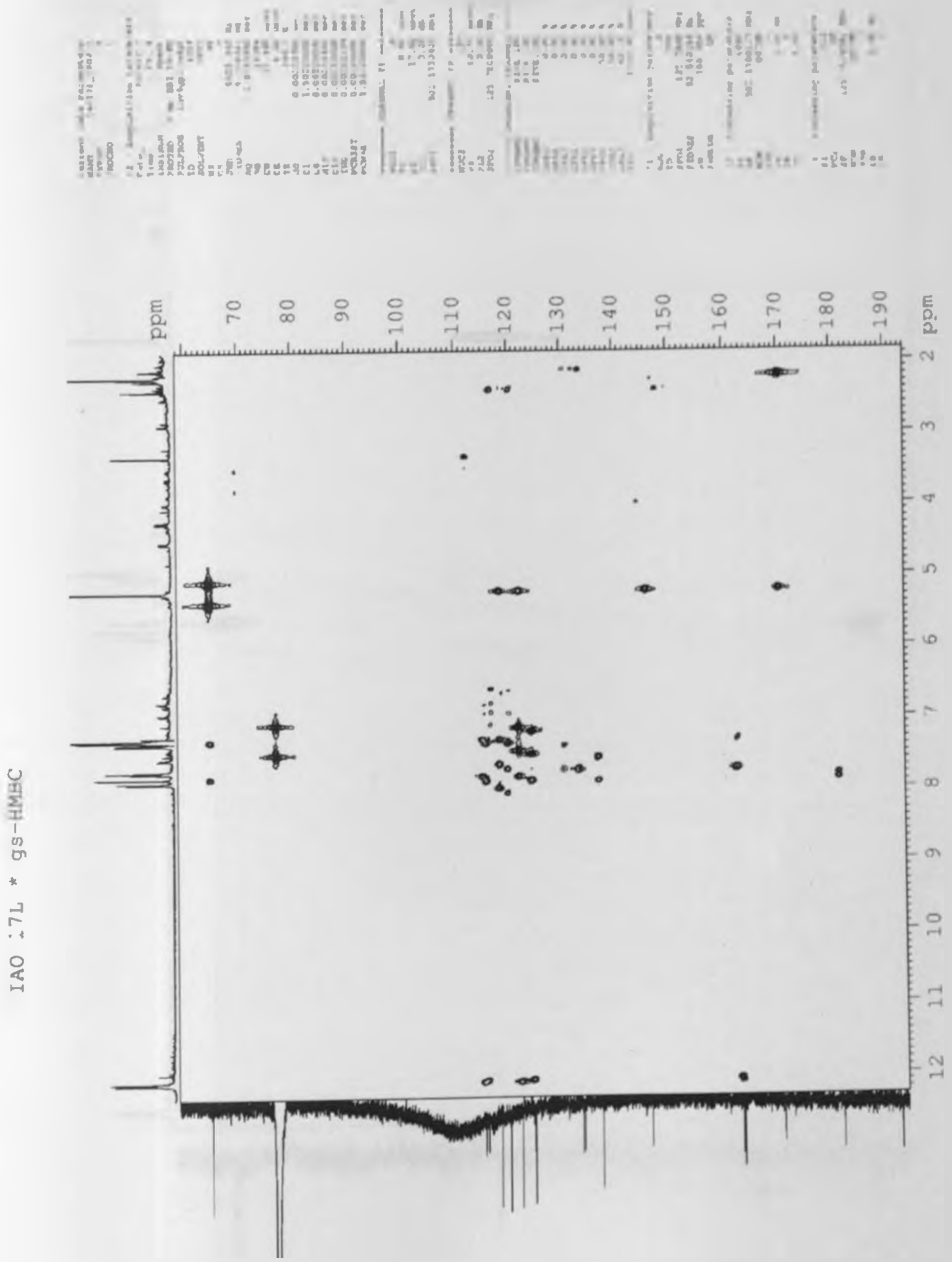
HMBC SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)



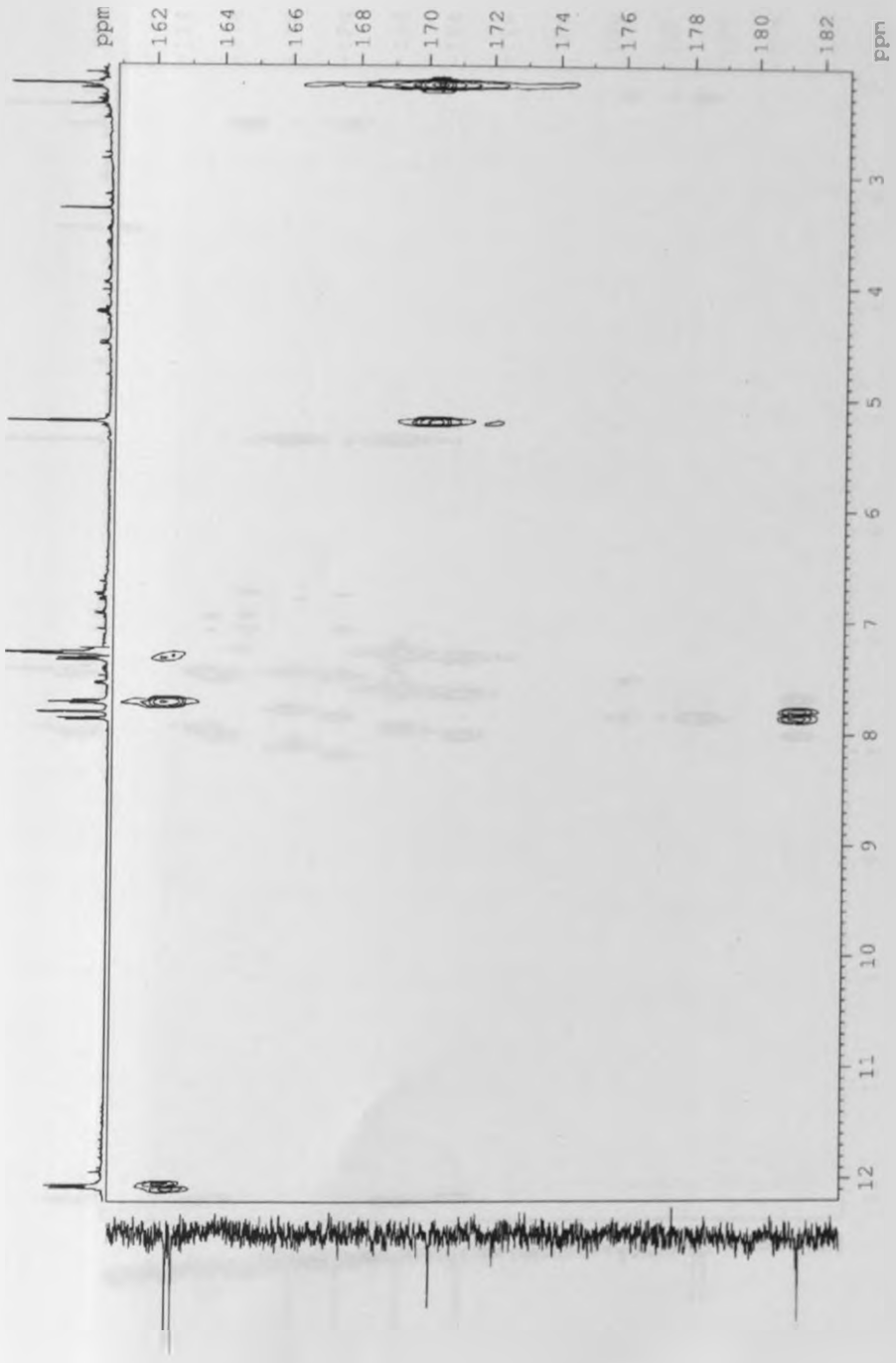
^{1}H NMR SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, 500 MHz)



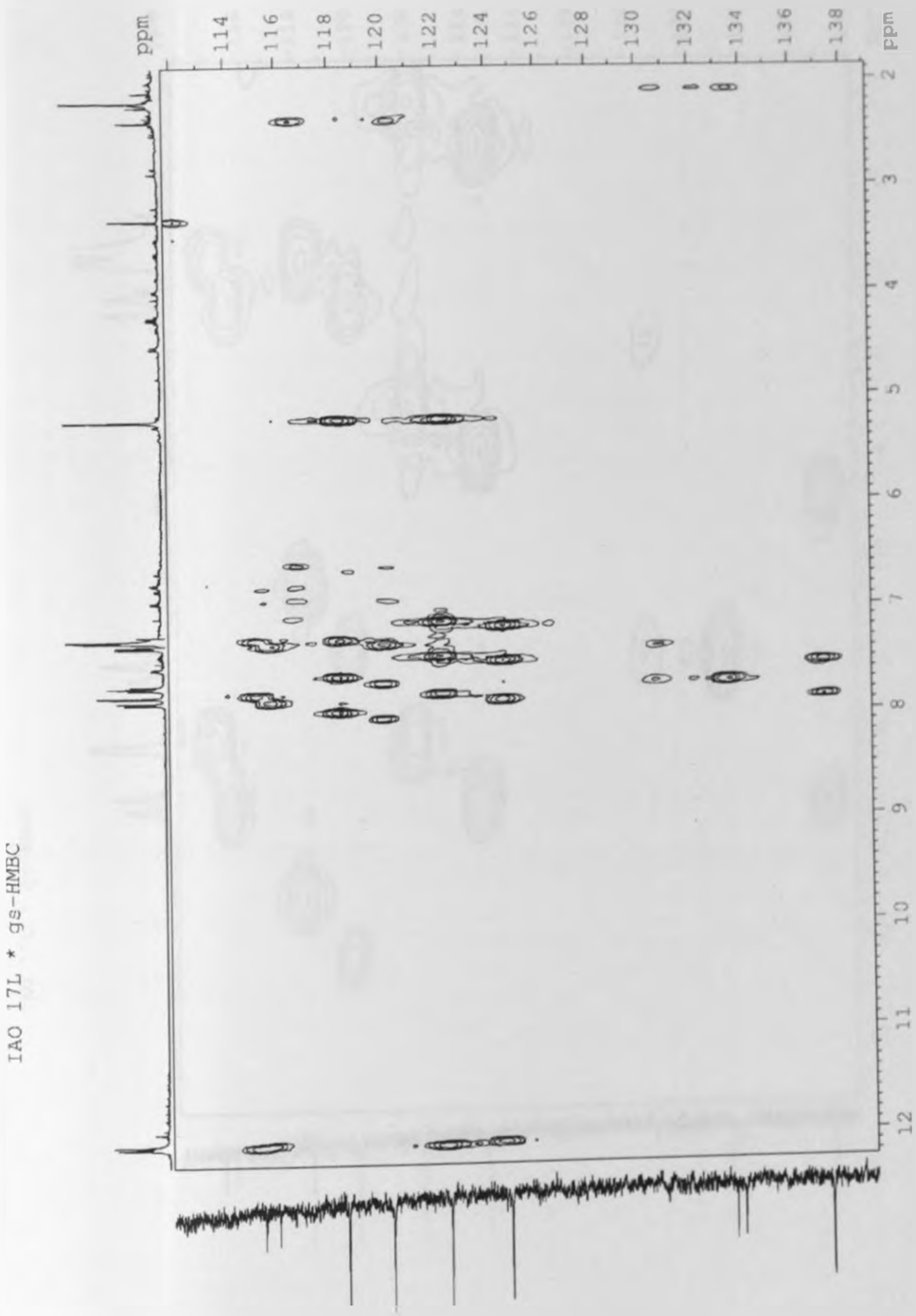
HMBC SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)



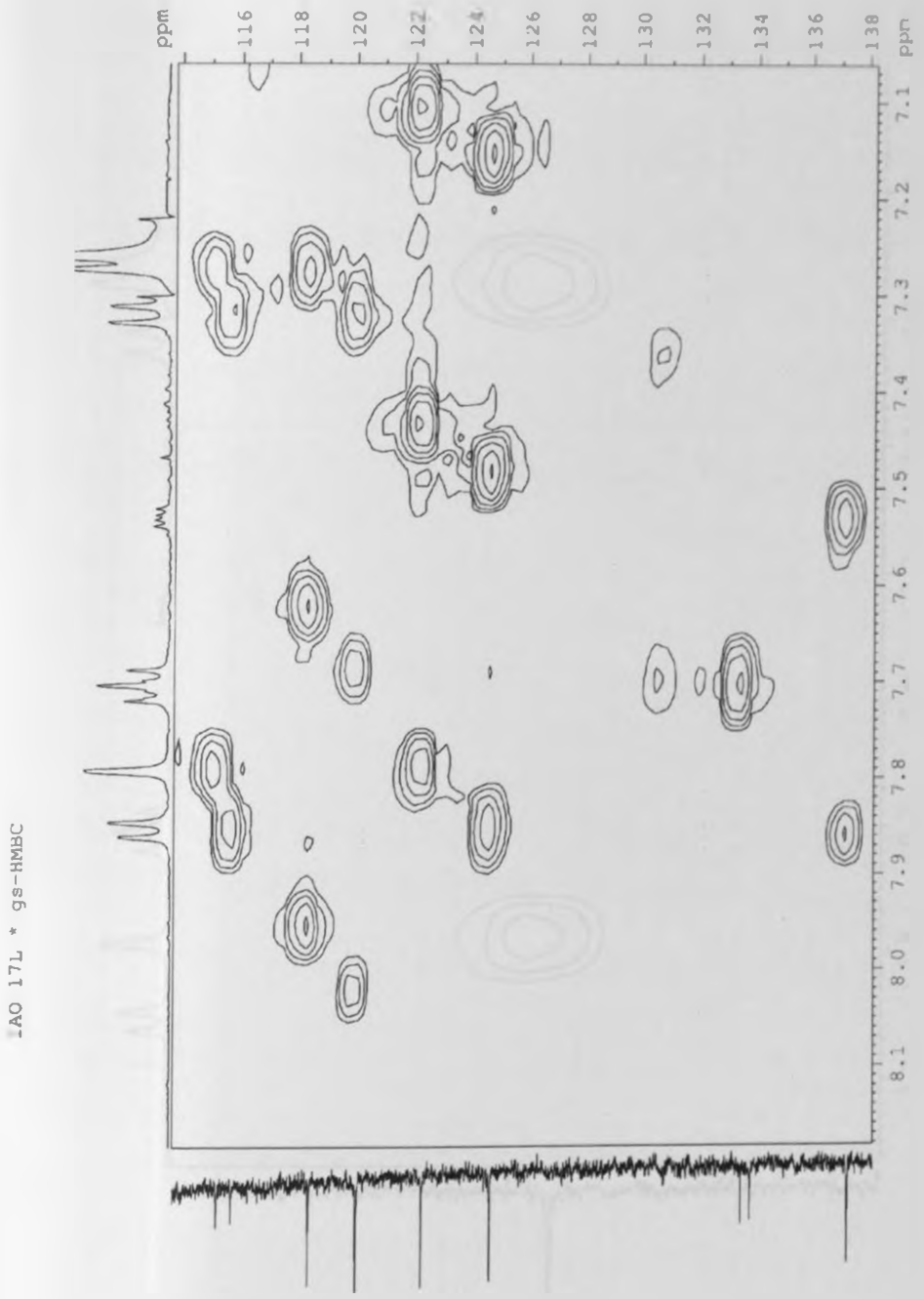
HMBC SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)



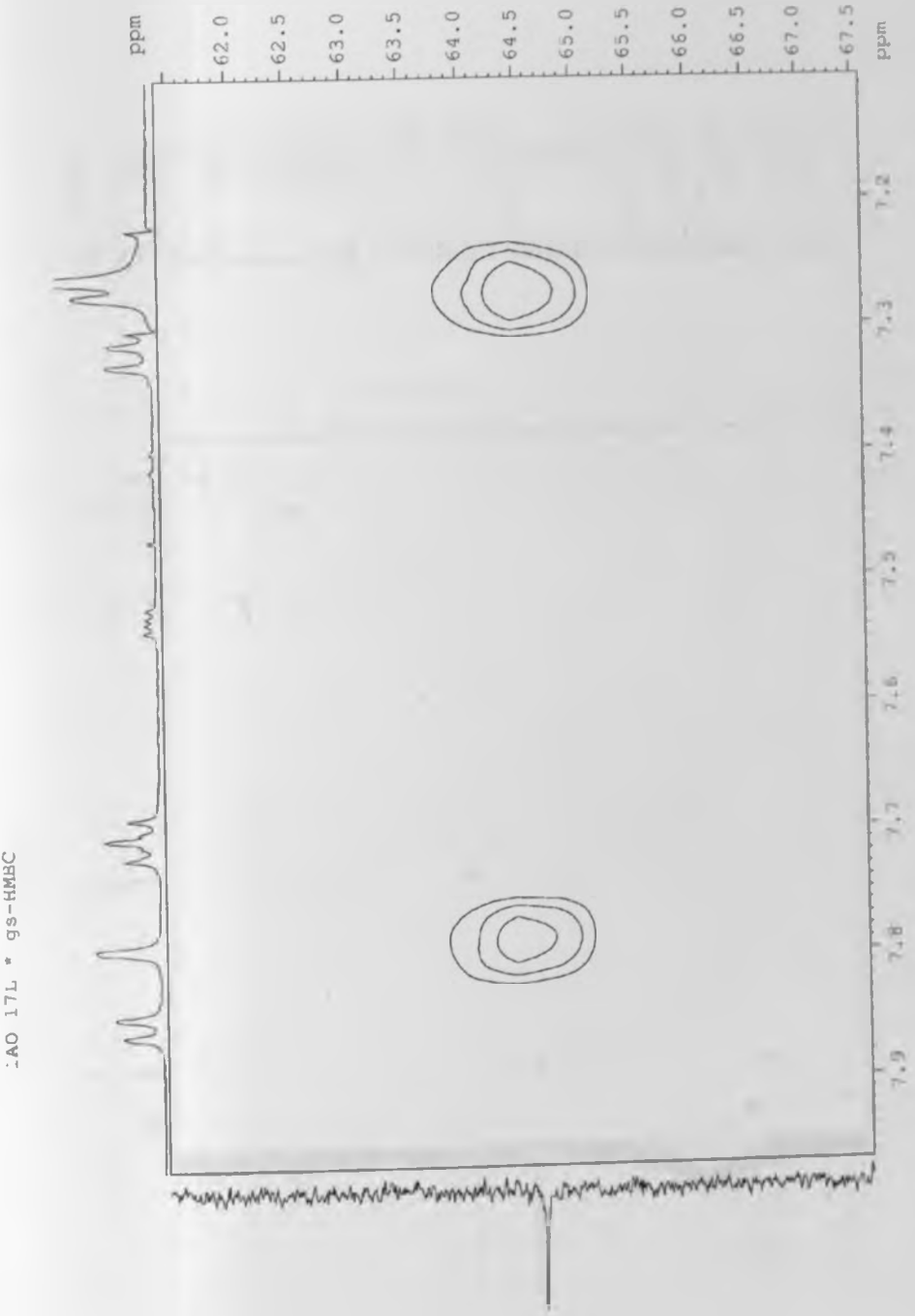
HMBC SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, ^{1}H-500 and ^{13}C-125 MHz)



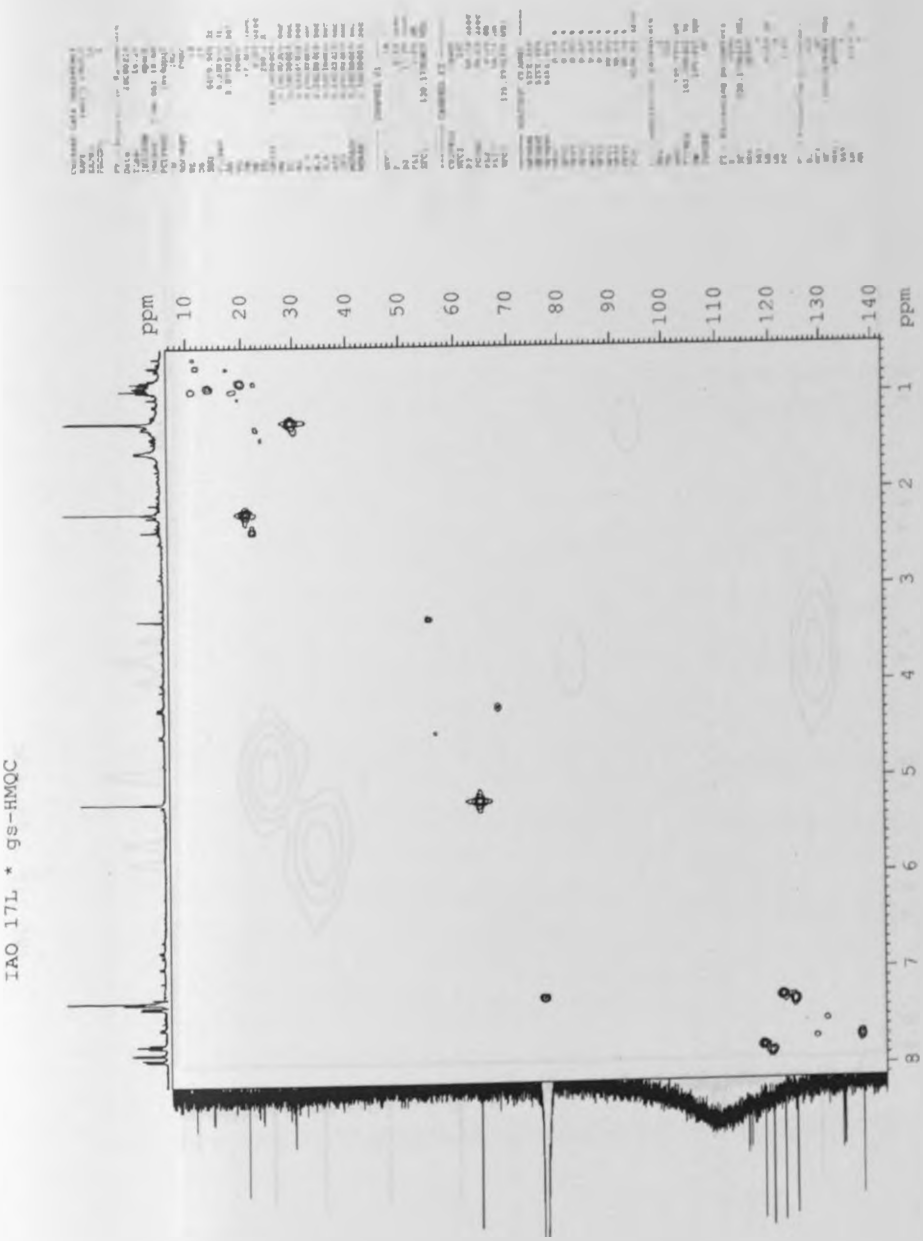
MBC SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 Hz)



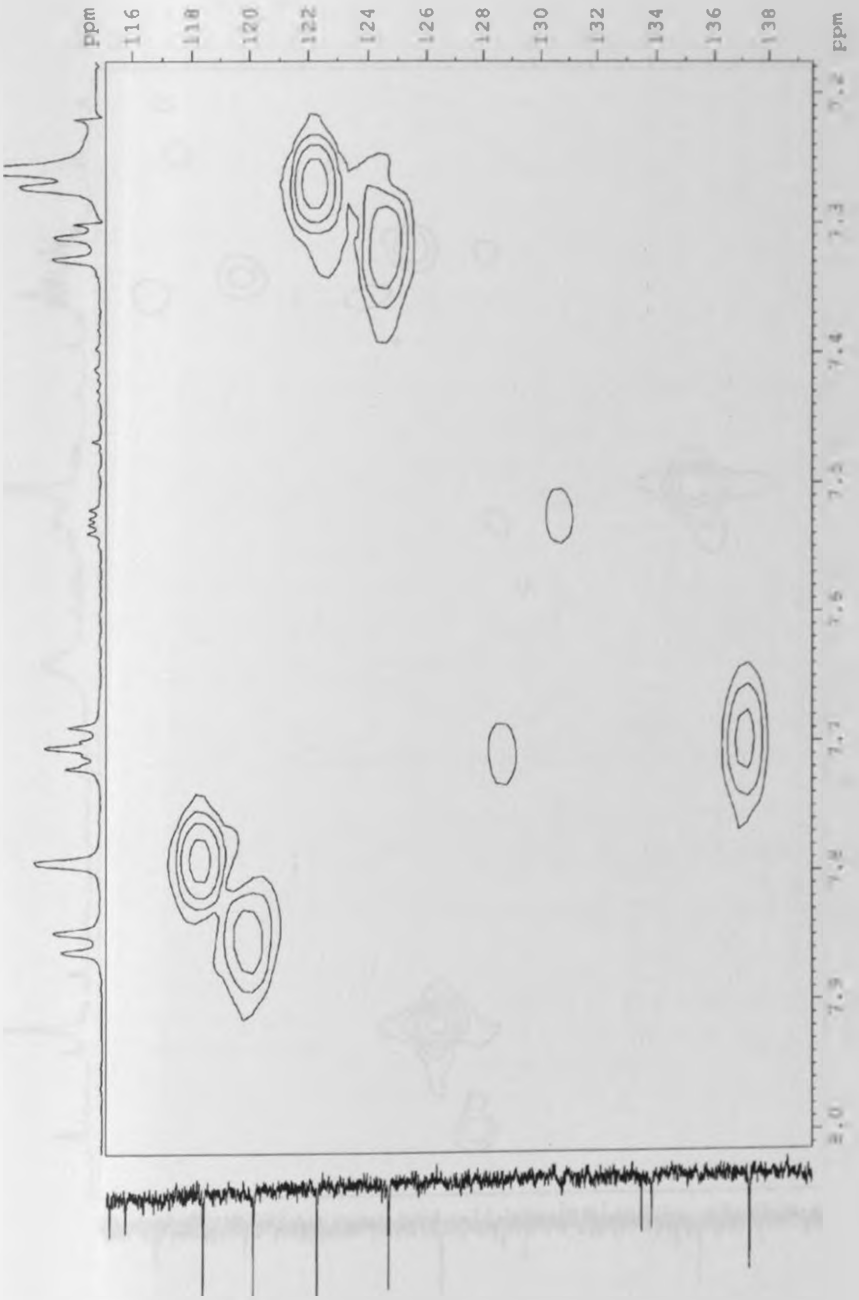
HMBC SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)



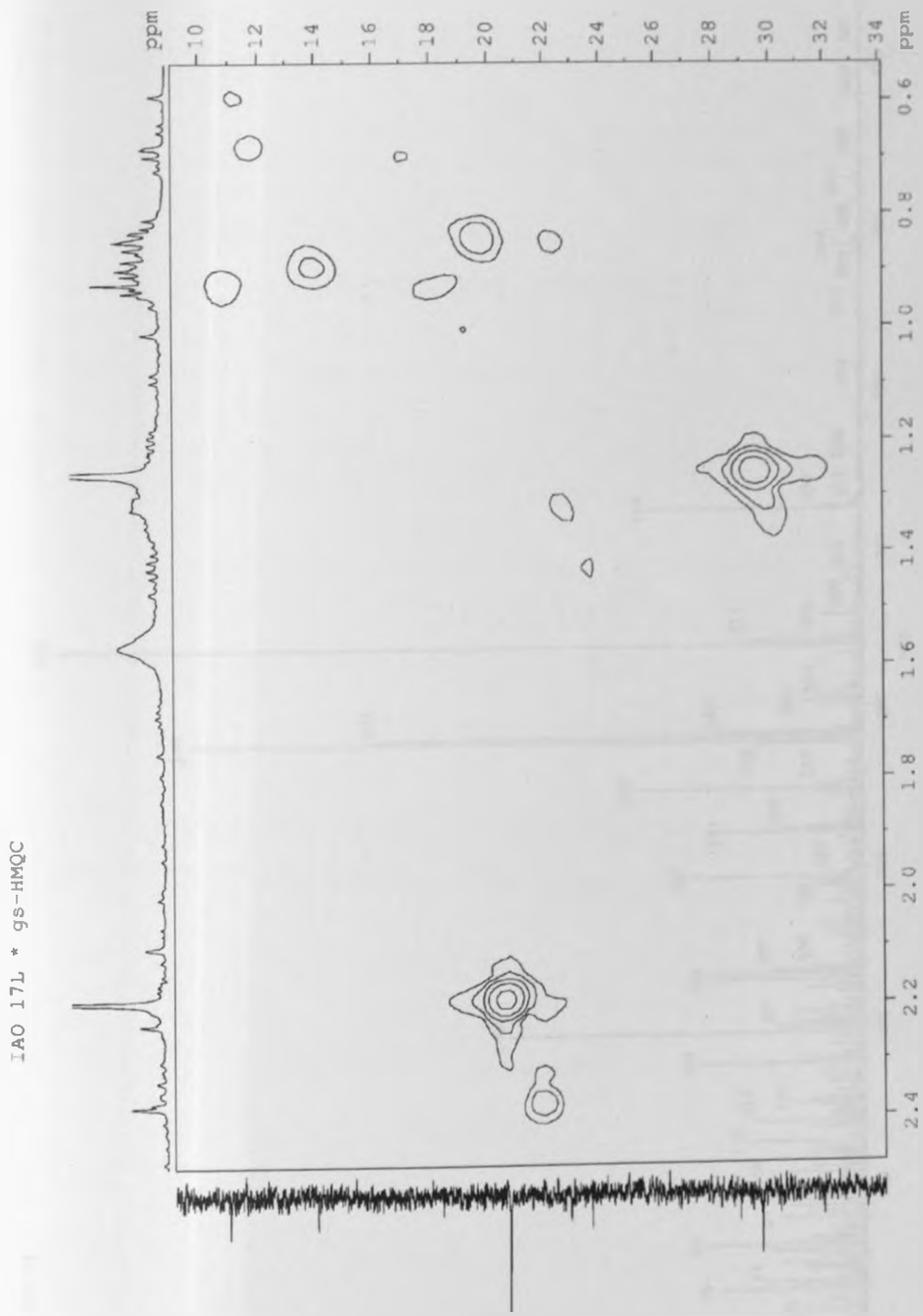
HMQC SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)



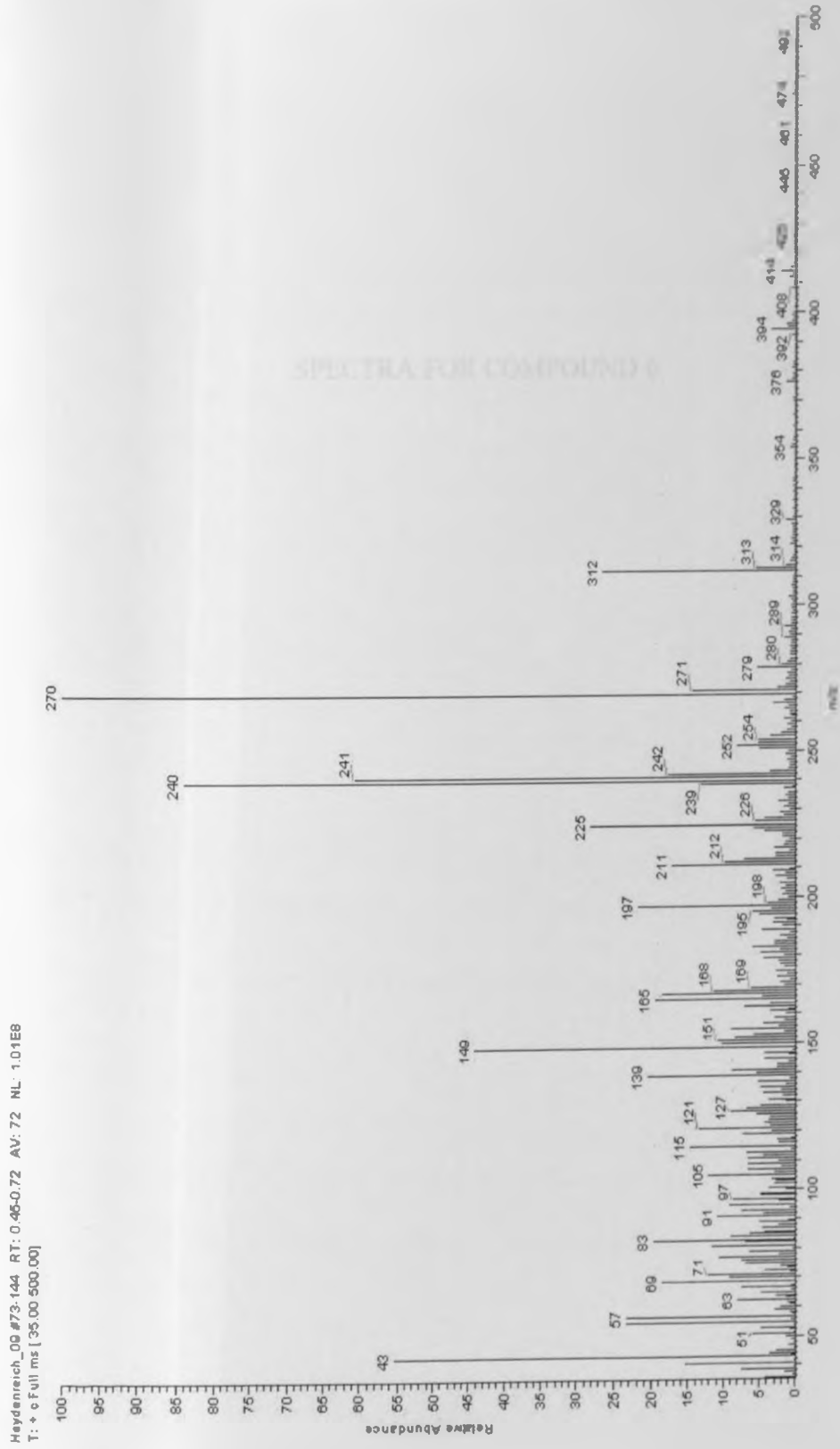
HMQC SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)



HMQC SPECTRUM FOR COMPOUND 5 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)

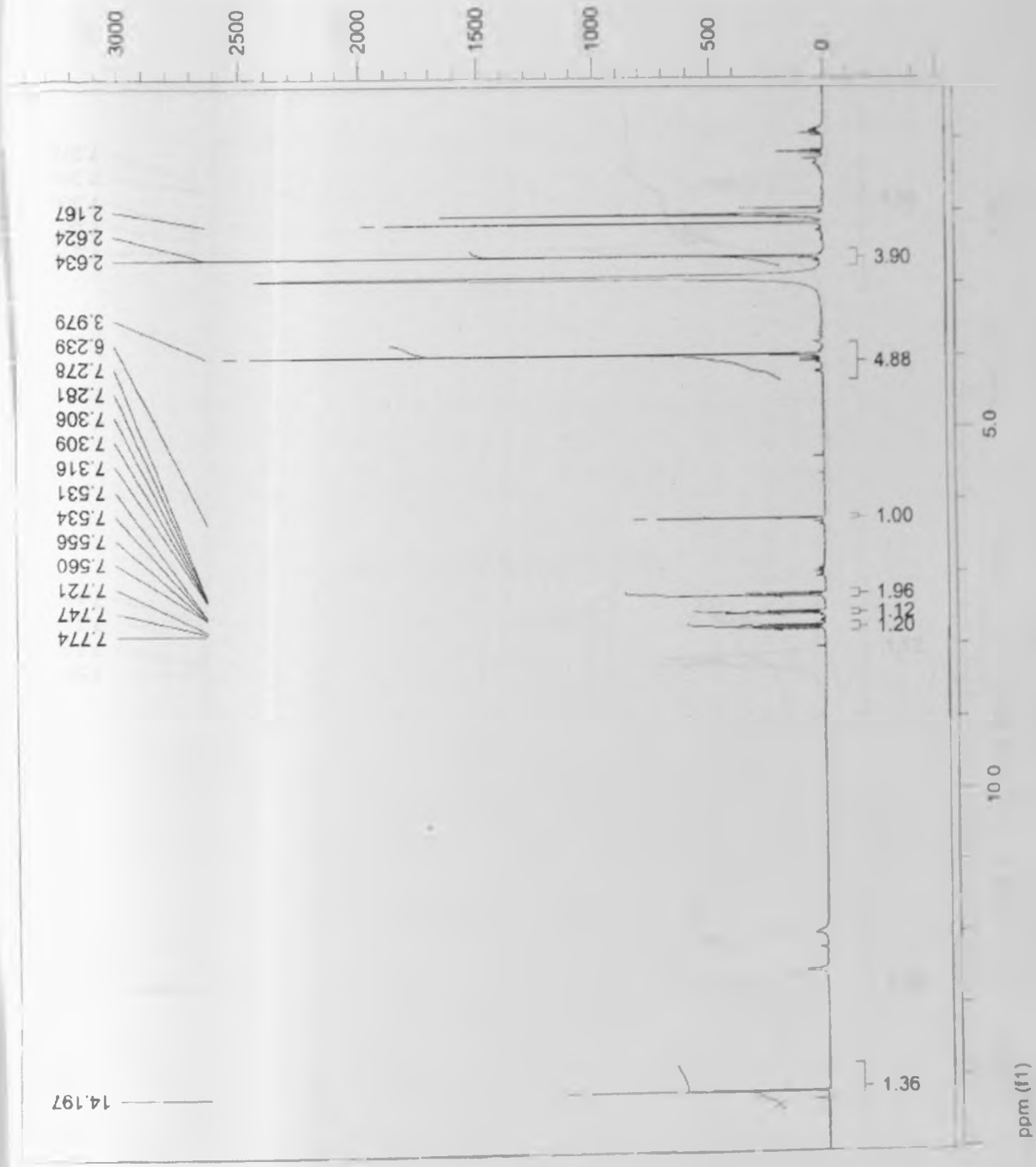


MS FOR COMPOUND 5

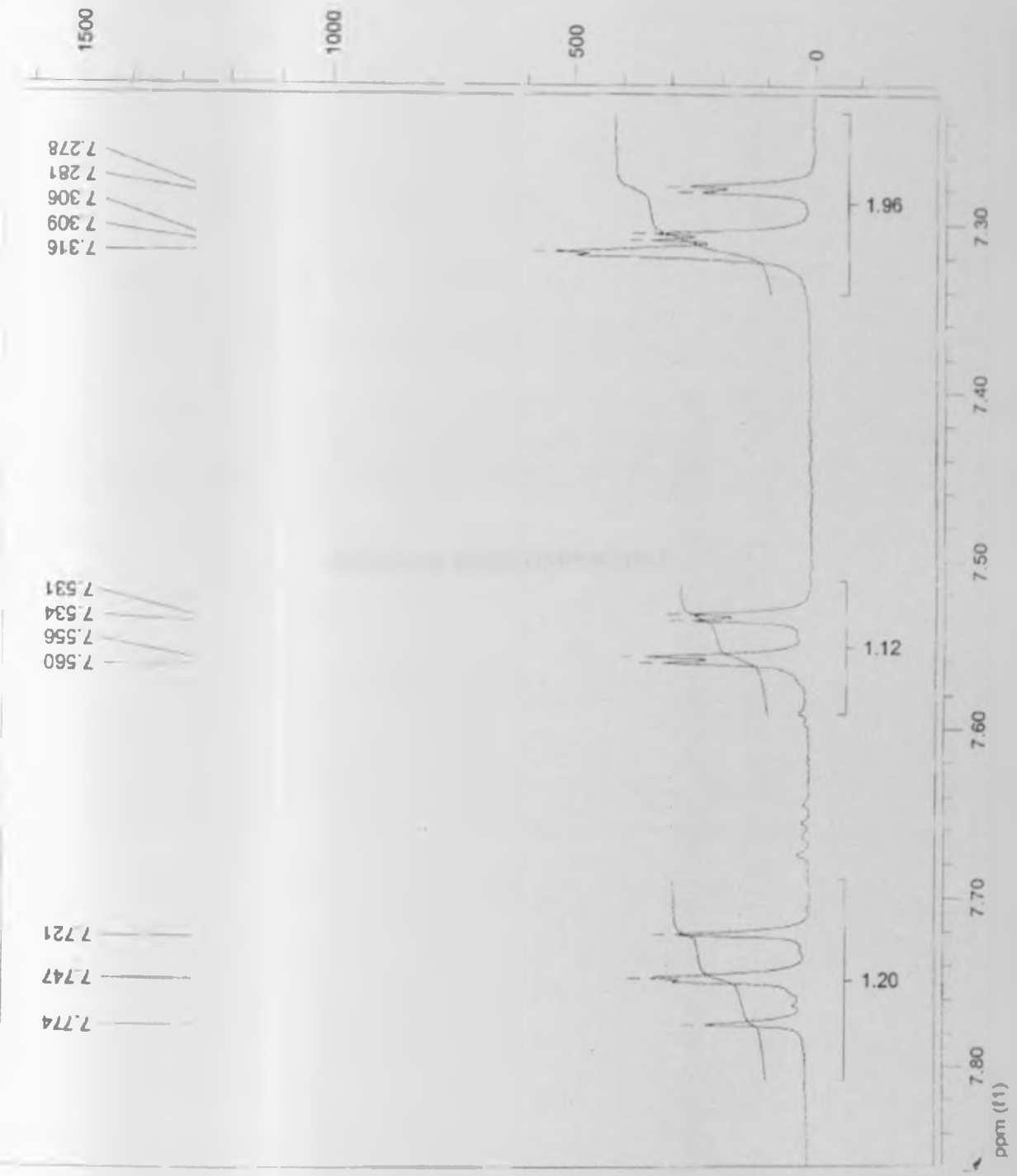


SPECTRA FOR COMPOUND 6

H NMR SPECTRUM FOR COMPOUND 6 (SOLVENT: ACETONE-d_6, 300 MHz)

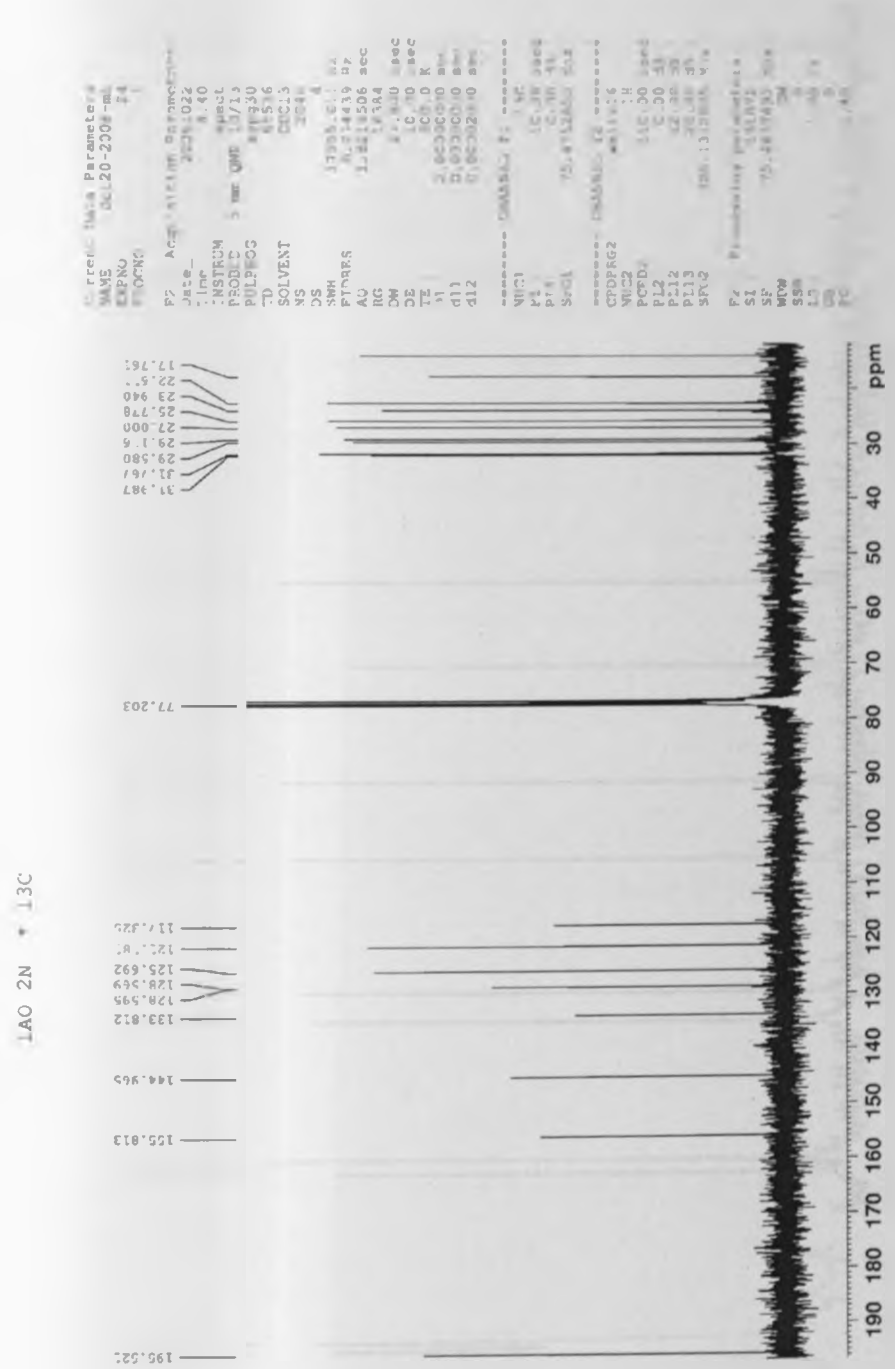


^{1}H NMR SPECTRUM FOR COMPOUND 6 (SOLVENT: ACETONE-d_6, 300 MHz)

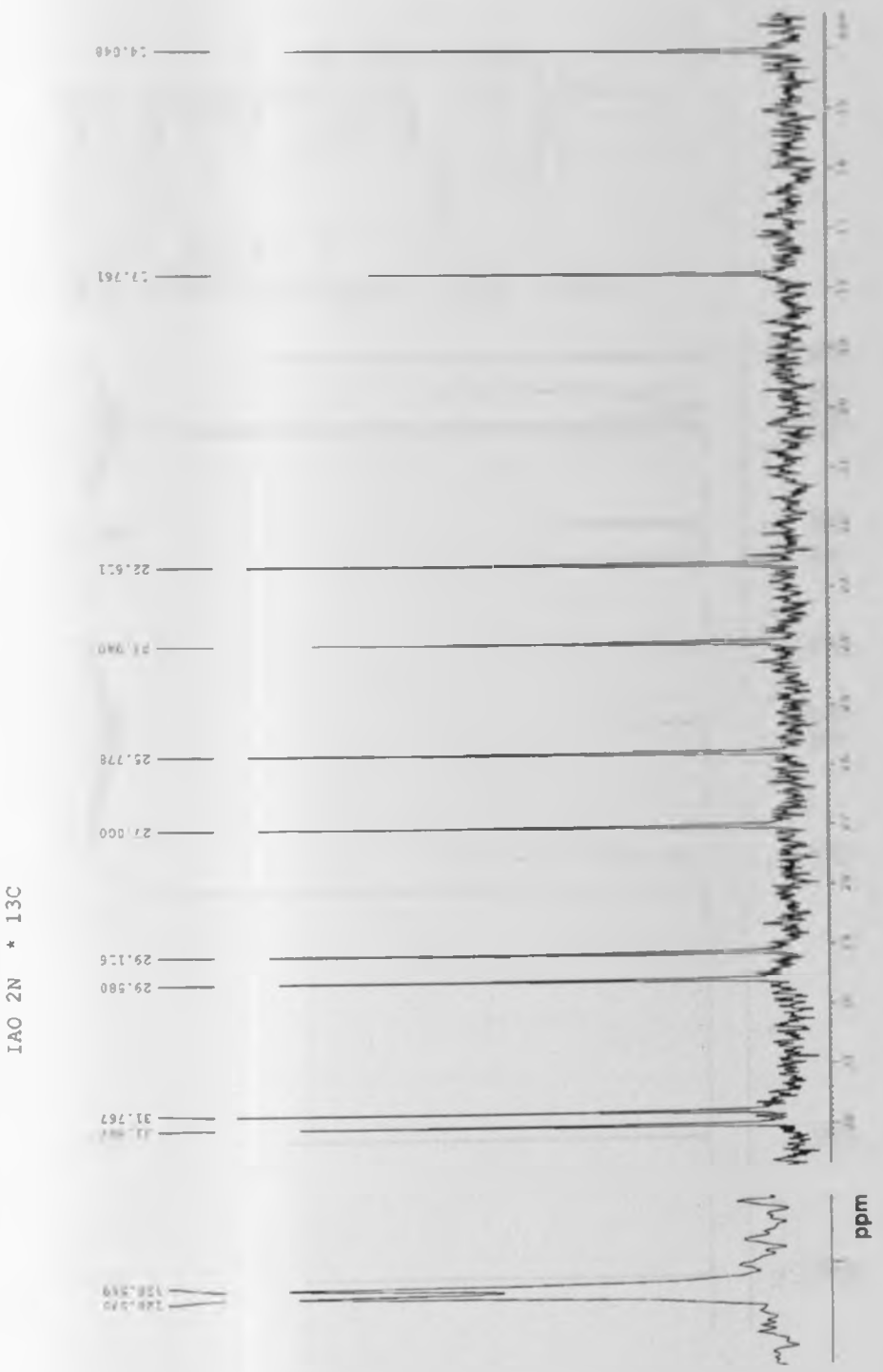


SPECTRA FOR COMPOUND 7

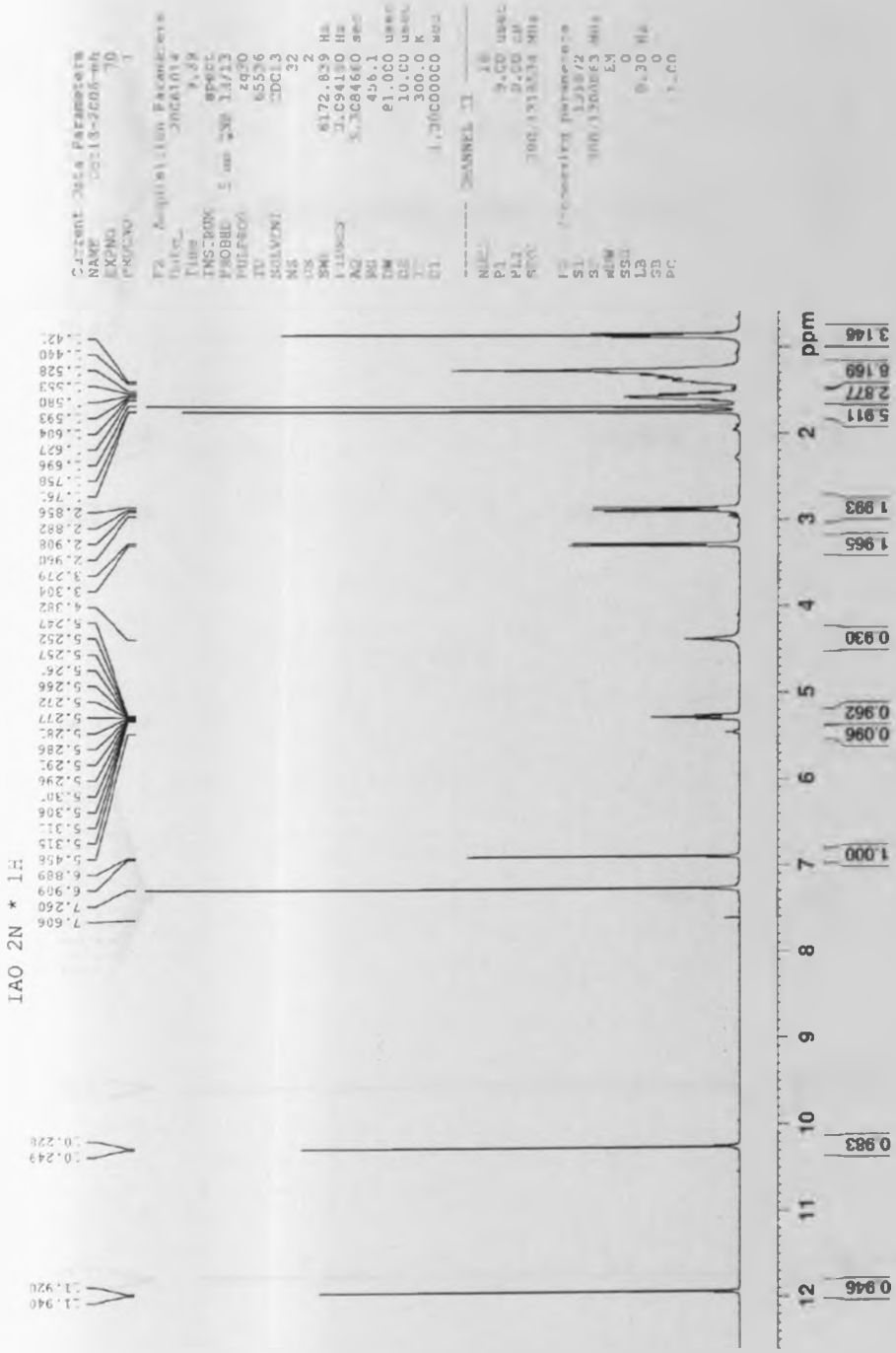
^{13}C NMR SPECTRUM FOR COMPOUND 7 (SOLVENT; $CDCl_3$, 75 MHz)



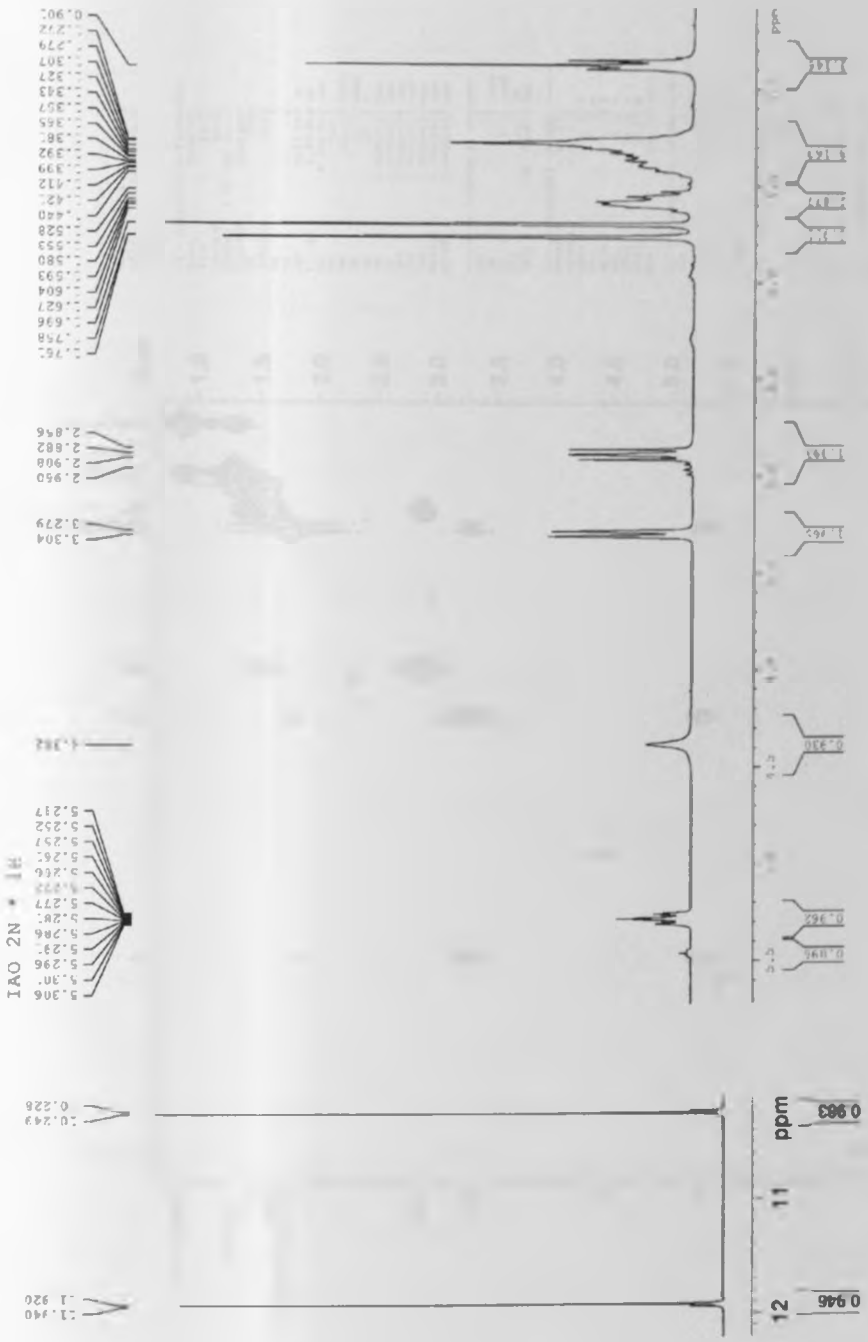
^{13}C NMR SPECTRUM FOR COMPOUND 7 (SOLVENT; $CDCl_3$, 75 MHz)



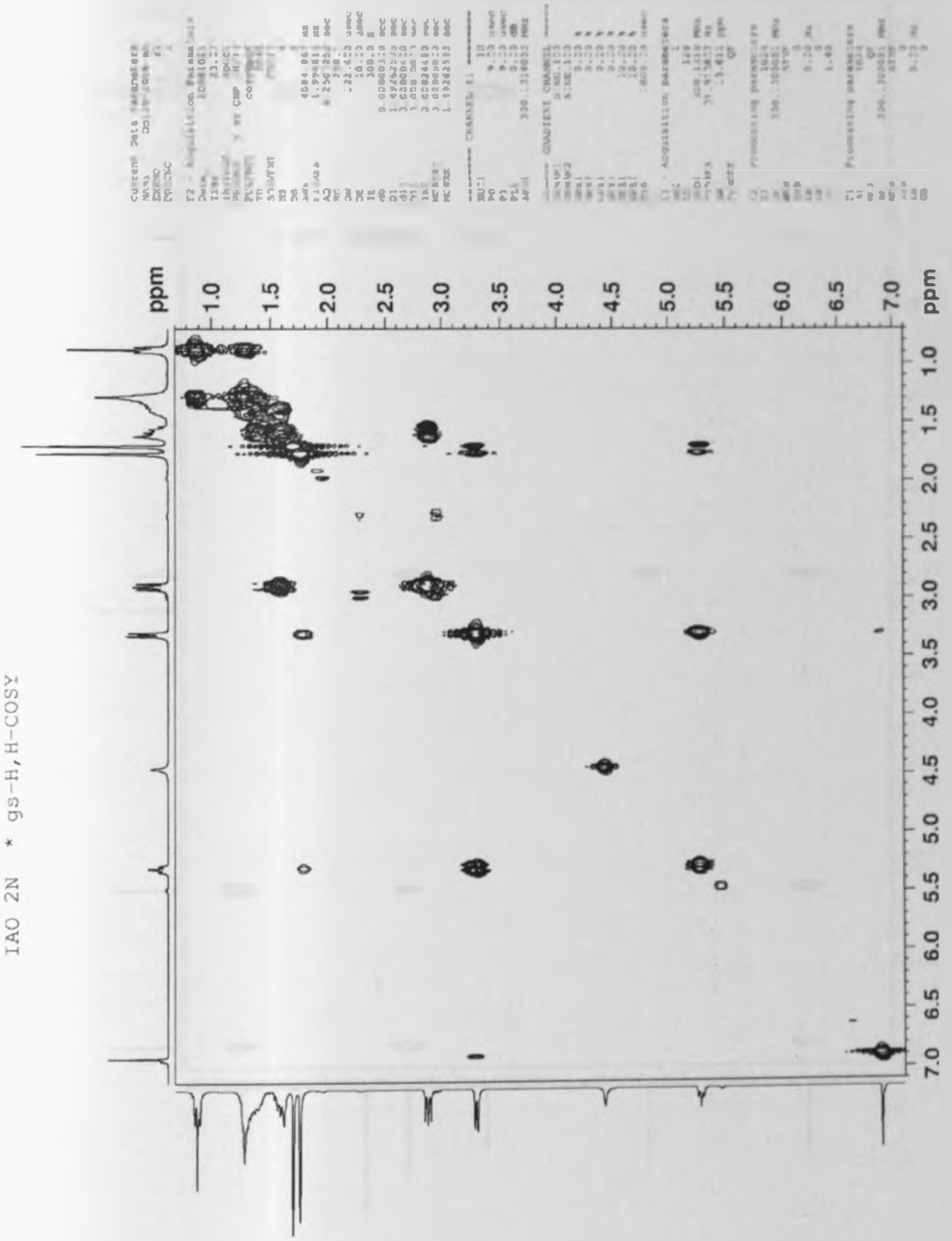
^{1}H NMR SPECTRUM FOR COMPOUND 7 (SOLVENT; $CDCl_3$, 300 MHz)



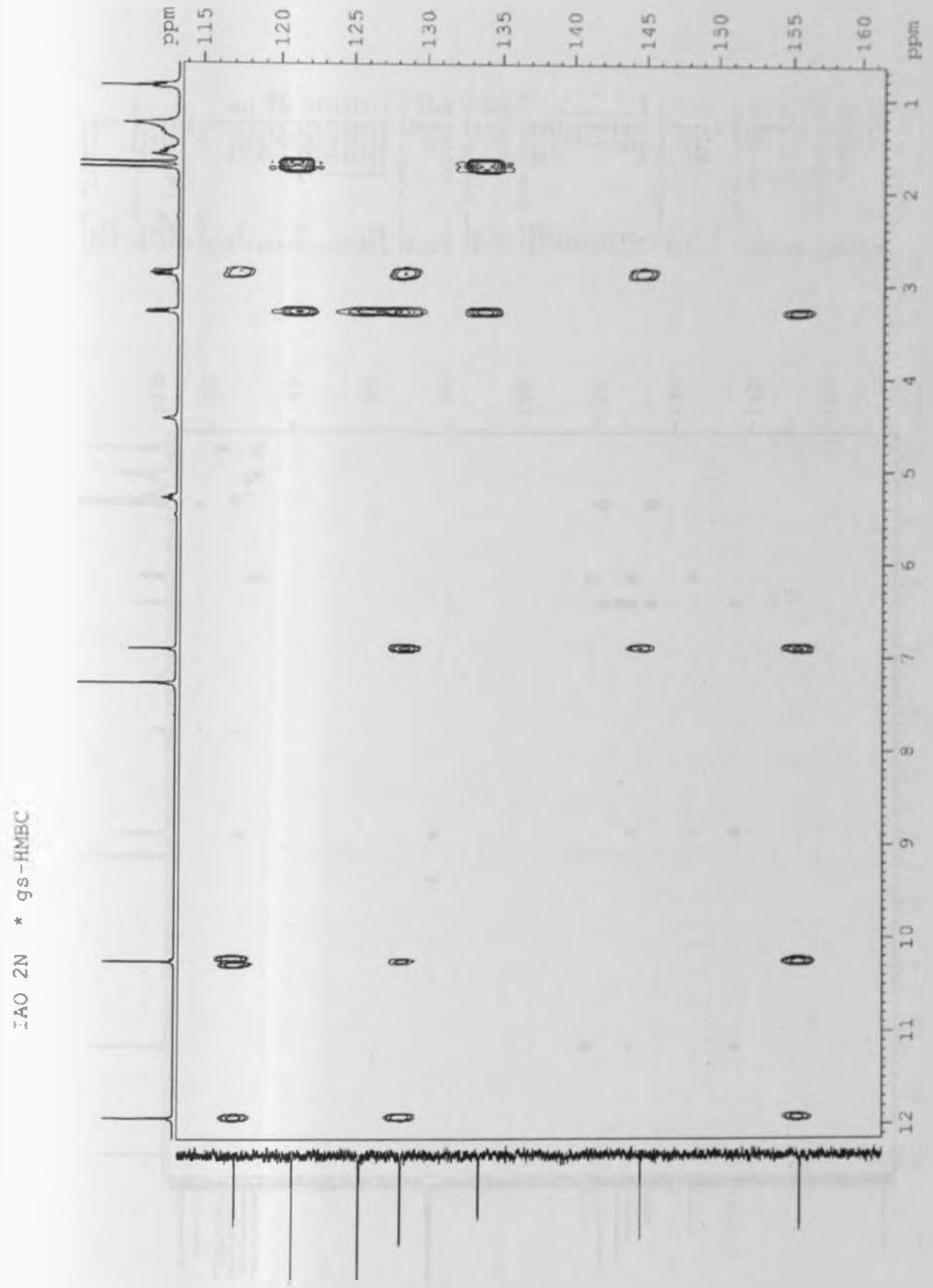
^{1}H NMR SPECTRUM FOR COMPOUND 7 (SOLVENT; $CDCl_3$, 300 MHz)



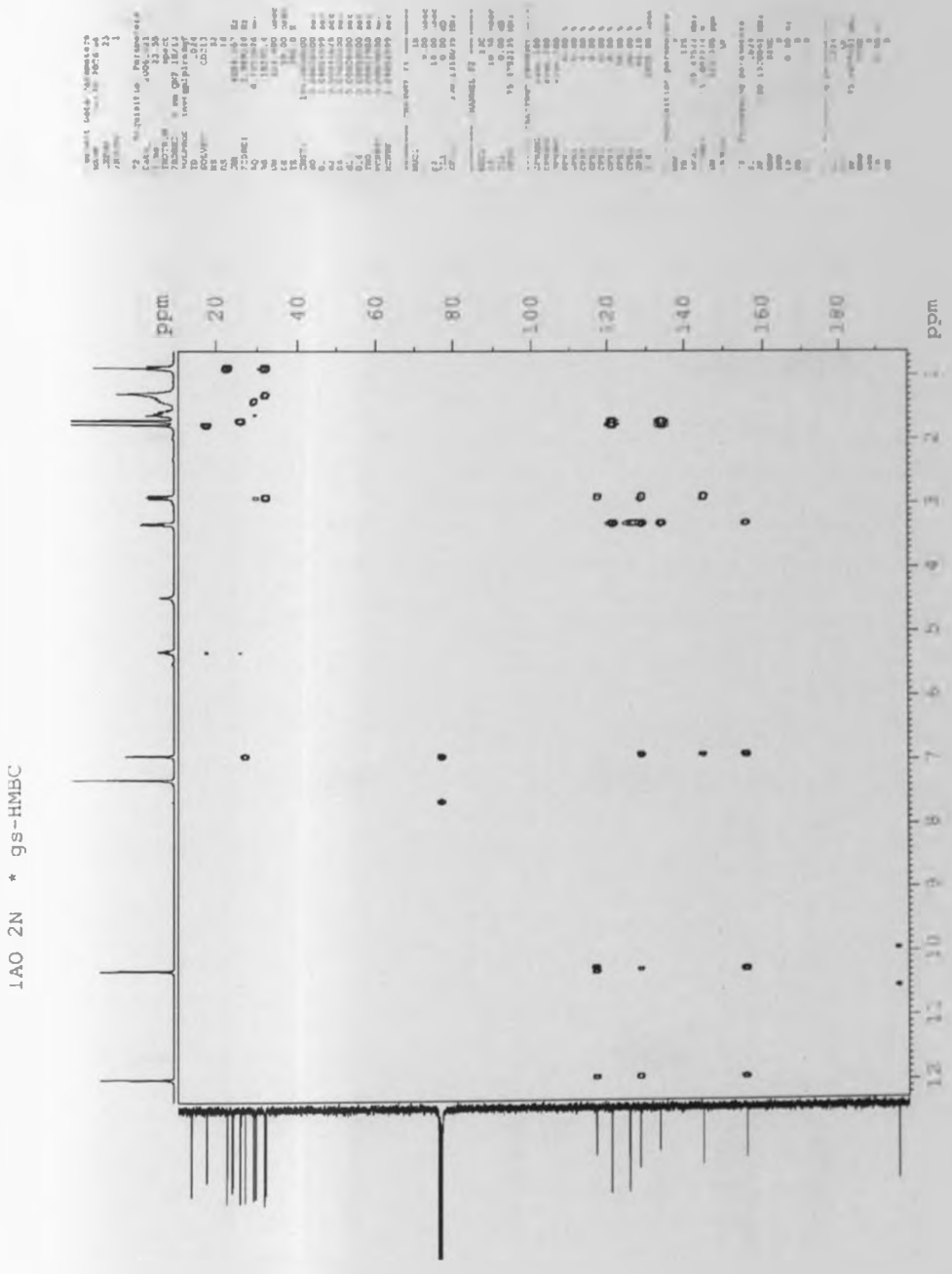
H, H-COSY SPECTRUM FOR COMPOUND 7 (SOLVENT; $CDCl_3$, 300 MHz)



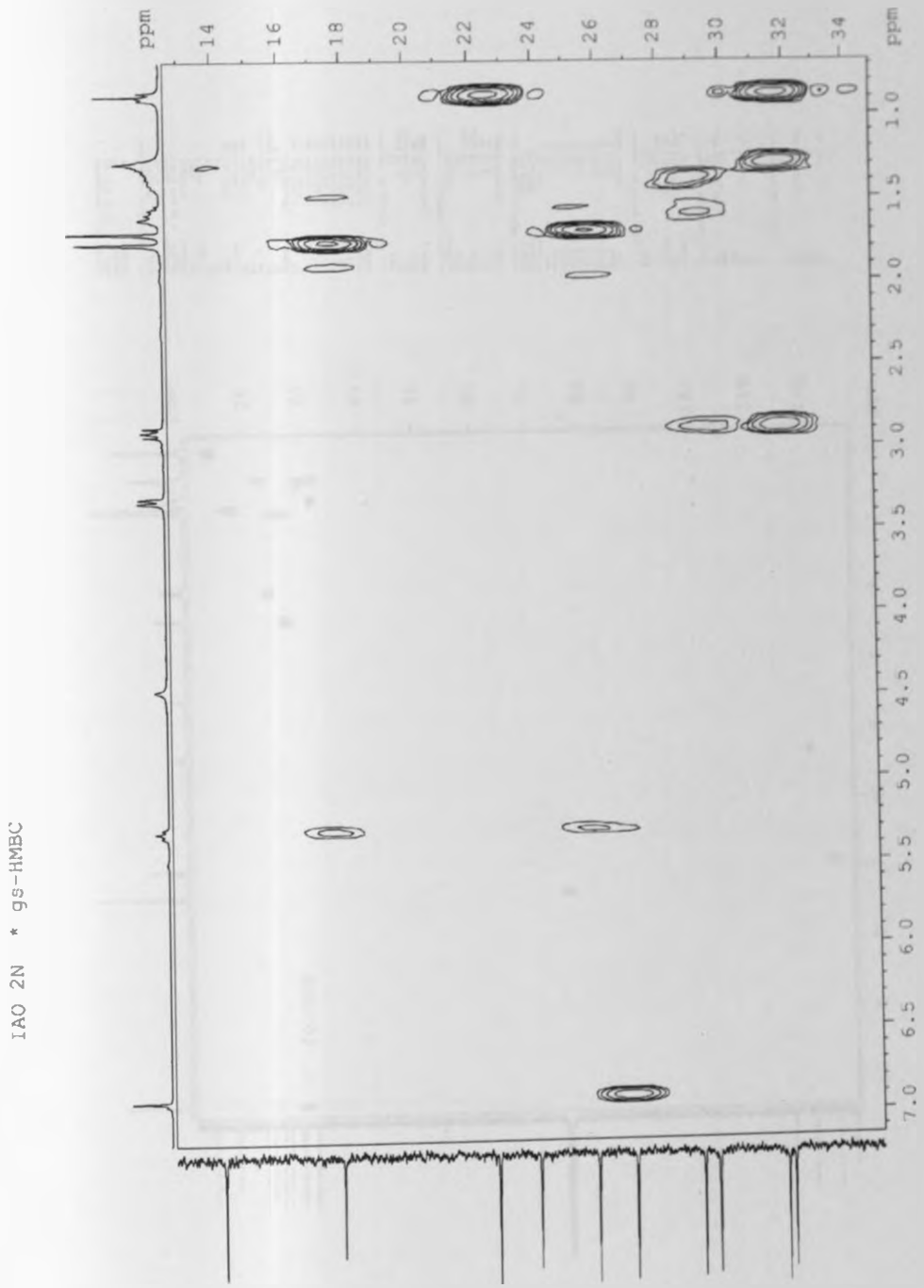
HMBC SPECTRUM FOR COMPOUND 7 (SOLVENT; $CDCl_3$, ^1H-300 and ^{13}C-75 MHz)



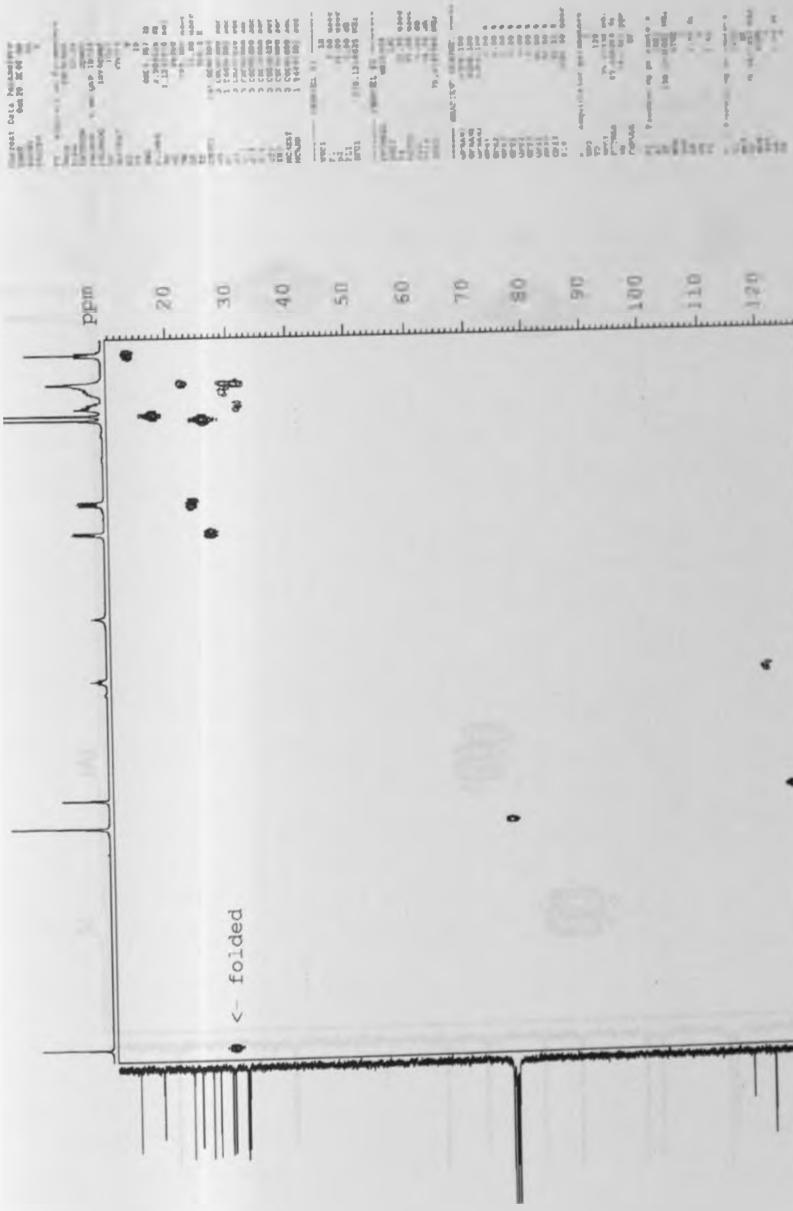
HMBC SPECTRUM FOR COMPOUND 7 (SOLVENT; $CDCl_3$, ^1H-300 and ^{13}C-75 MHz)



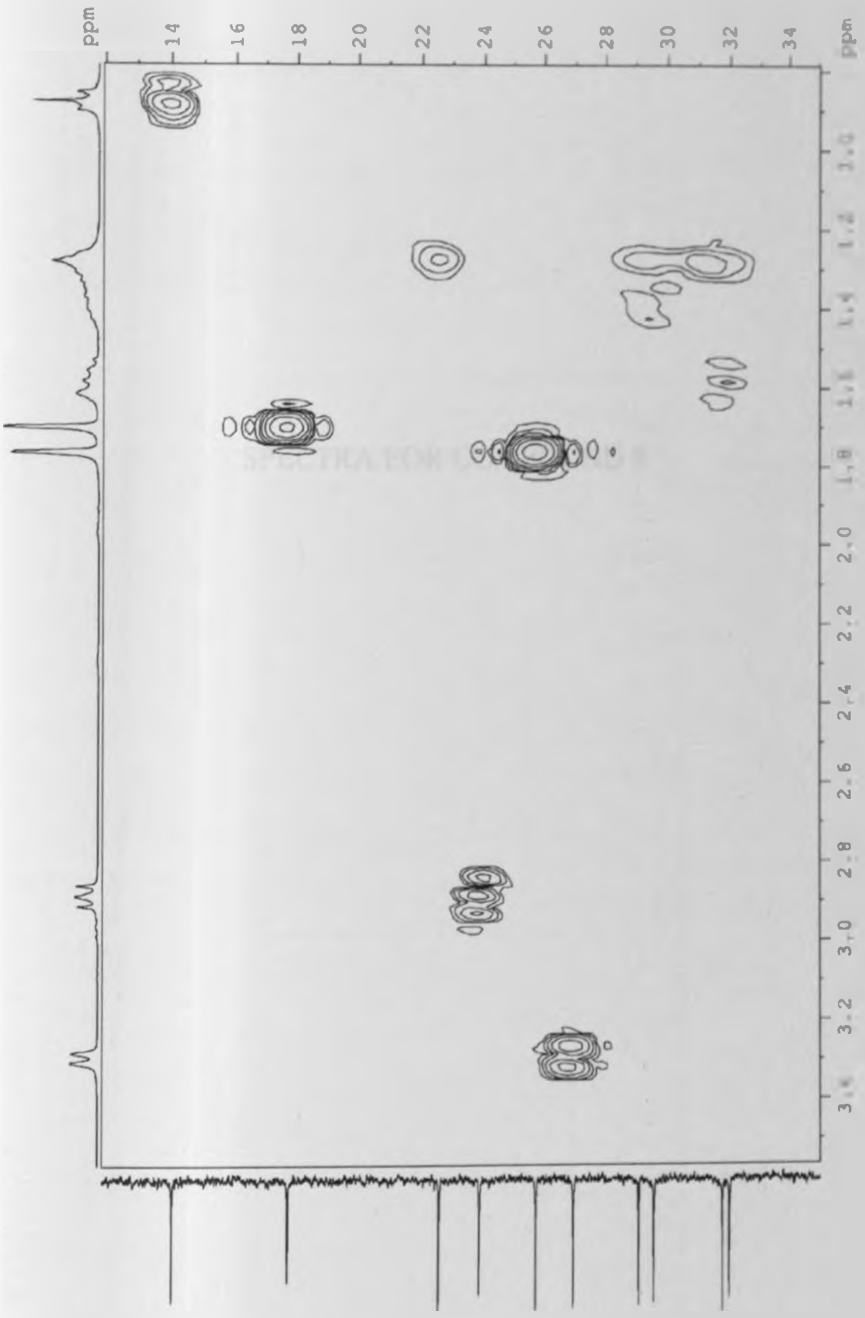
HMBC SPECTRUM FOR COMPOUND 7 (SOLVENT; $CDCl_3$, ^1H-300 and ^{13}C-75 MHz)



HMQC SPECTRUM FOR COMPOUND 7 (SOLVENT; $CDCl_3$, ^1H-300 and ^{13}C-75 MHz)

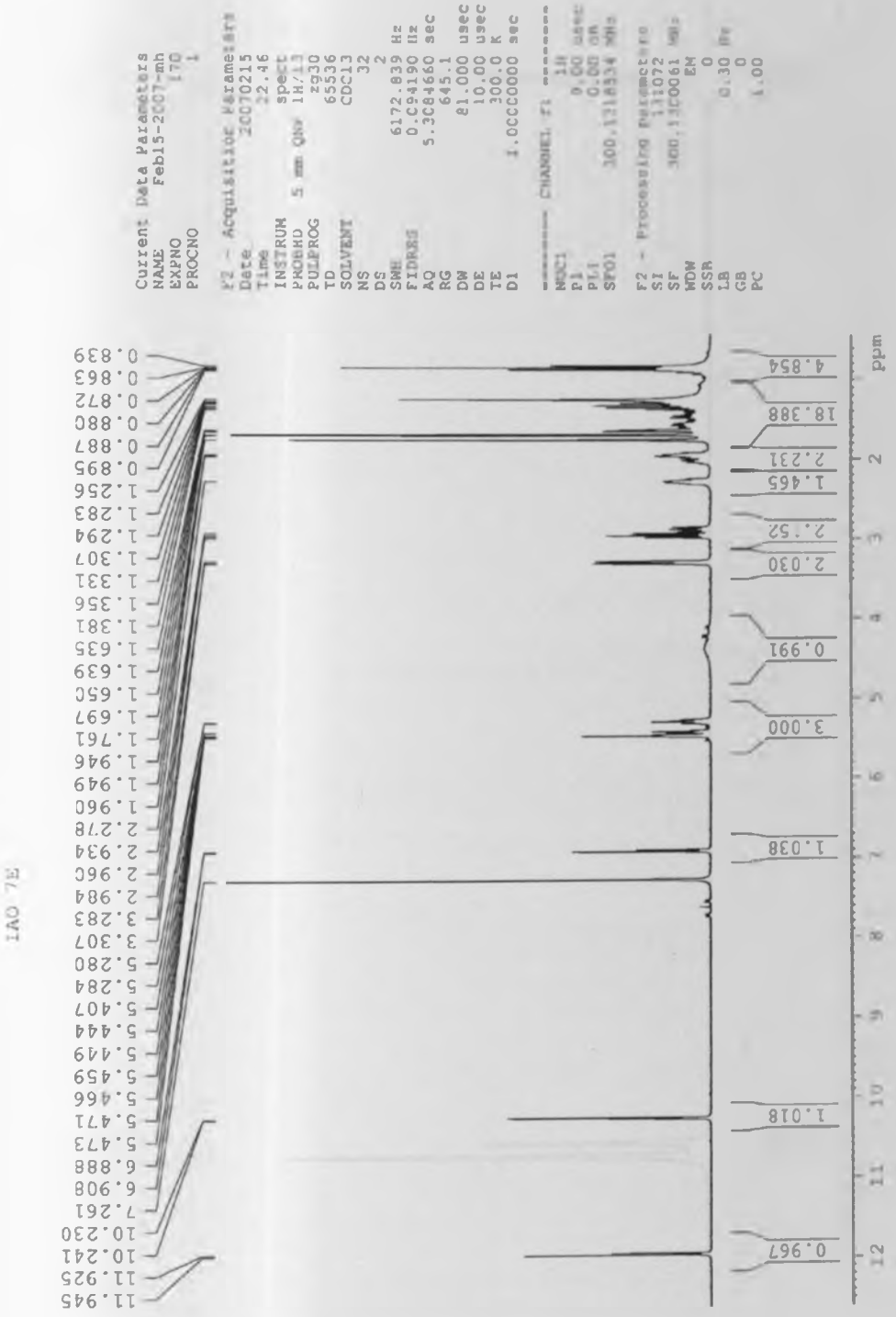


HMQC SPECTRUM FOR COMPOUND 7 (SOLVENT; $CDCl_3$, ^1H-300 and ^{13}C-75 MHz)

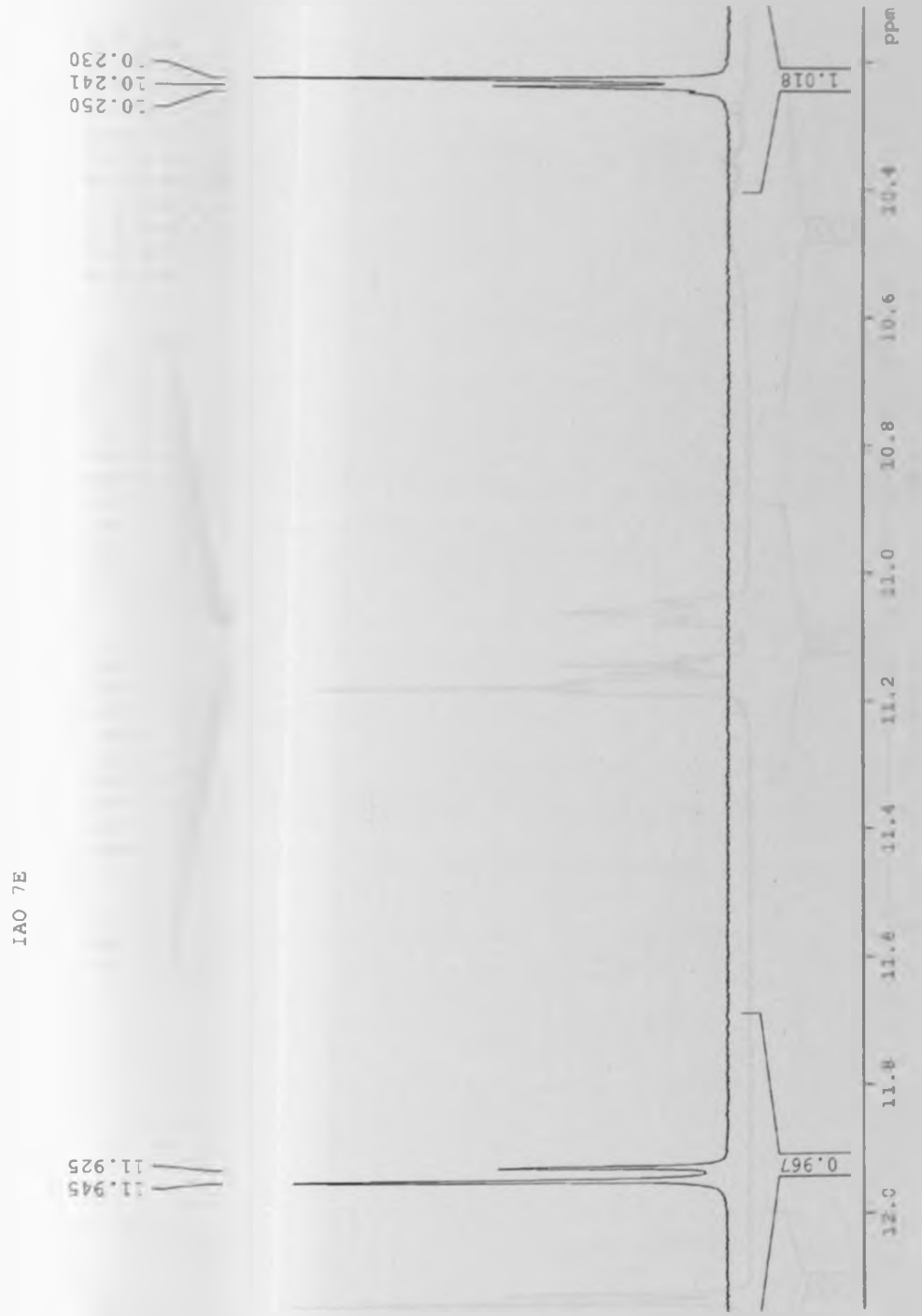


SPECTRA FOR COMPOUND 8

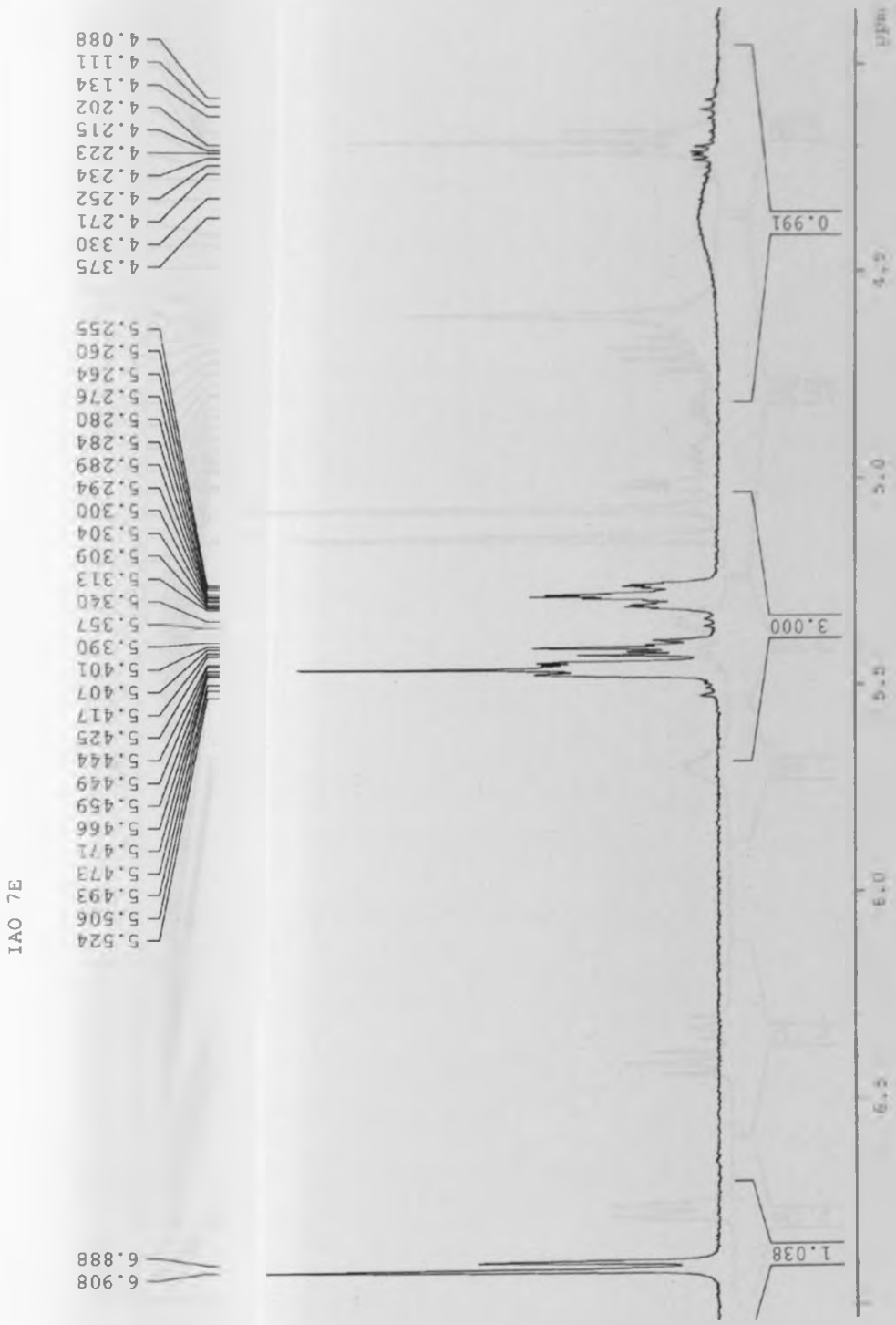
^{1}H NMR SPECTRUM FOR COMPOUND 8 (SOLVENT; $CDCl_3$, 300 MHz)



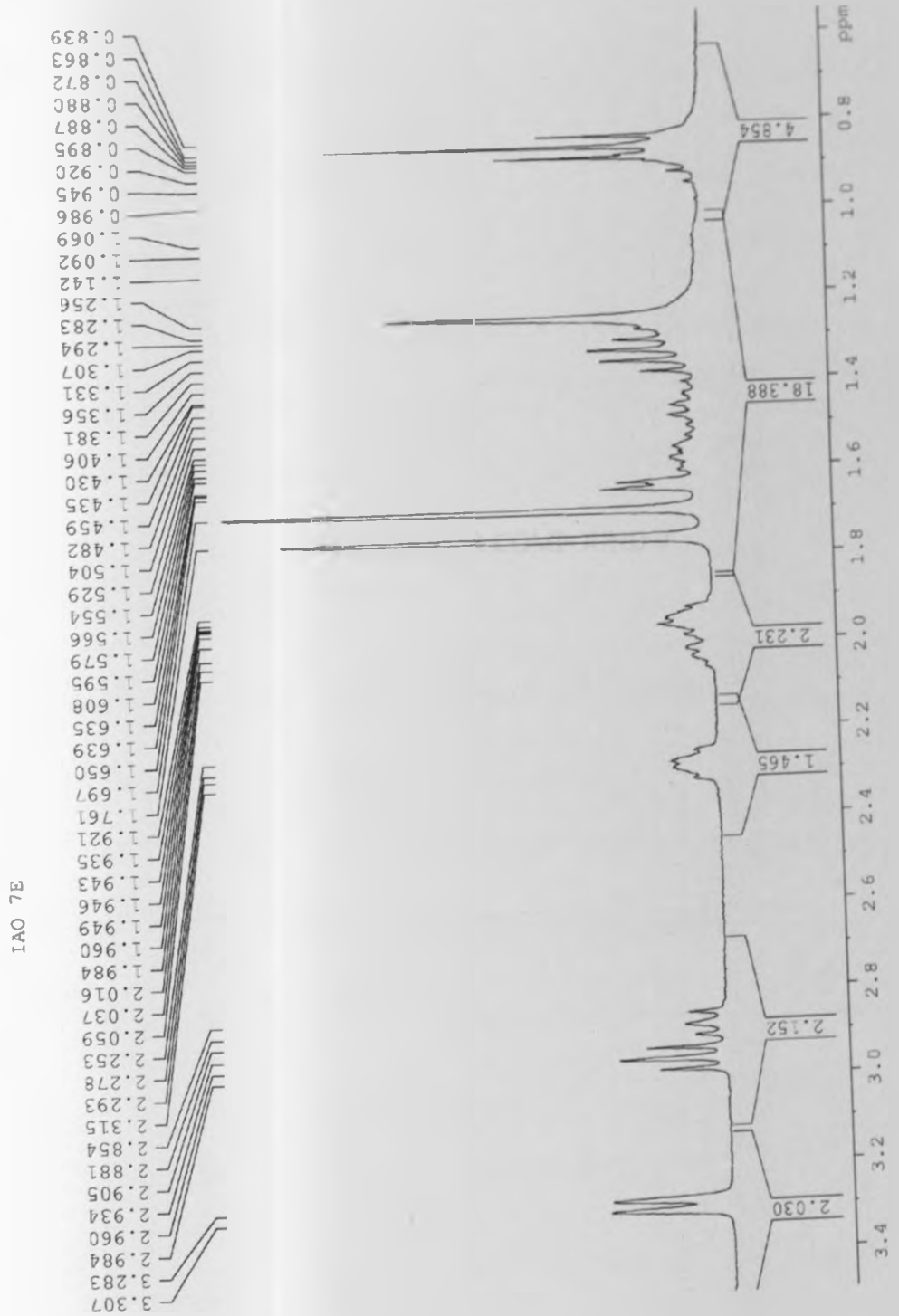
^{1}H NMR SPECTRUM FOR COMPOUND 8 (SOLVENT; $CDCl_3$, 300 MHz)



^{1}H NMR SPECTRUM FOR COMPOUND 8 (SOLVENT; $CDCl_3$, 300 MHz)

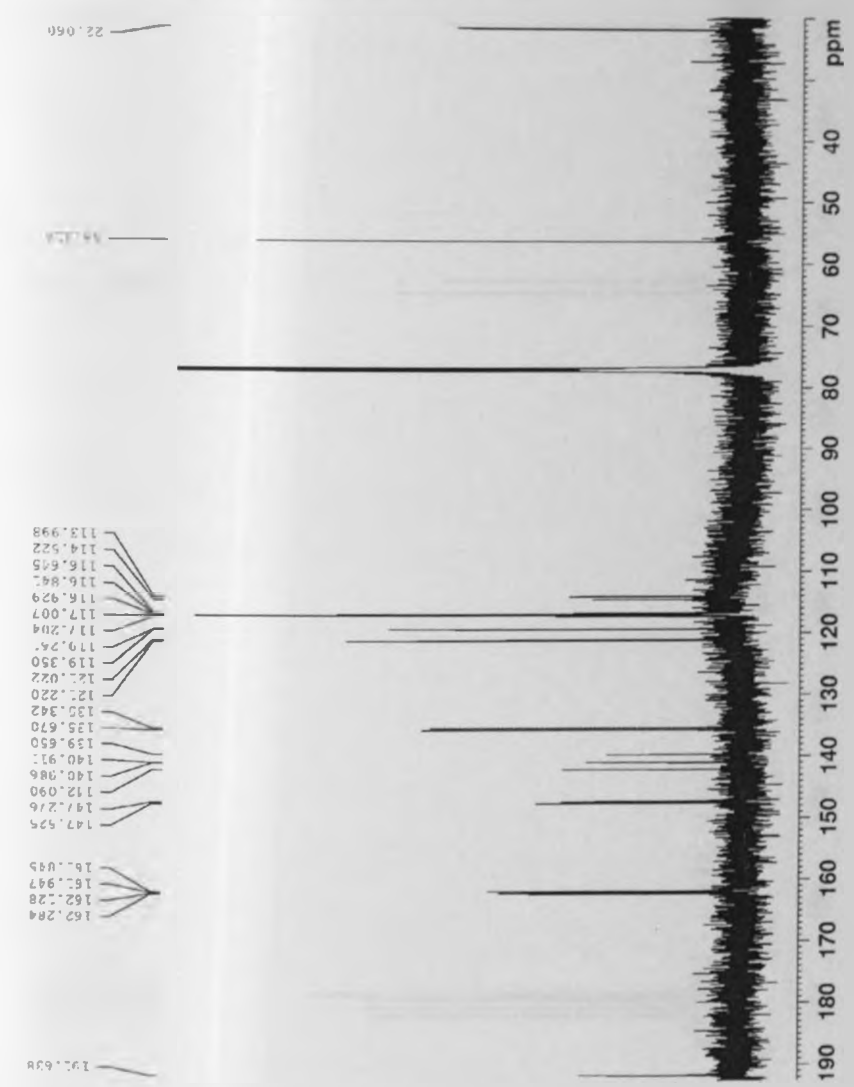


^{1}H NMR SPECTRUM FOR COMPOUND 8 (SOLVENT; $CDCl_3$, 300 MHz)

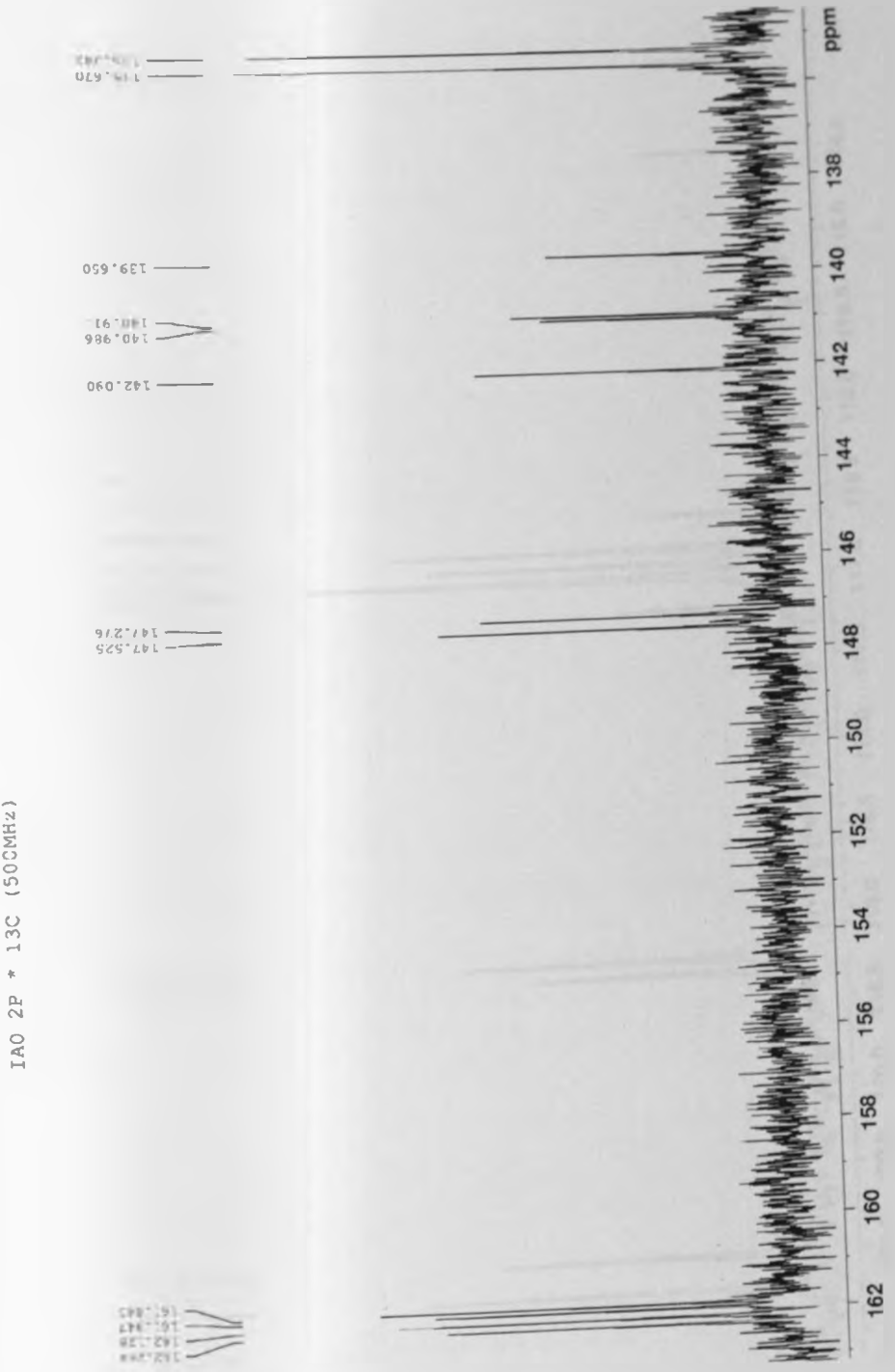


SPECTRA FOR COMPOUND 9

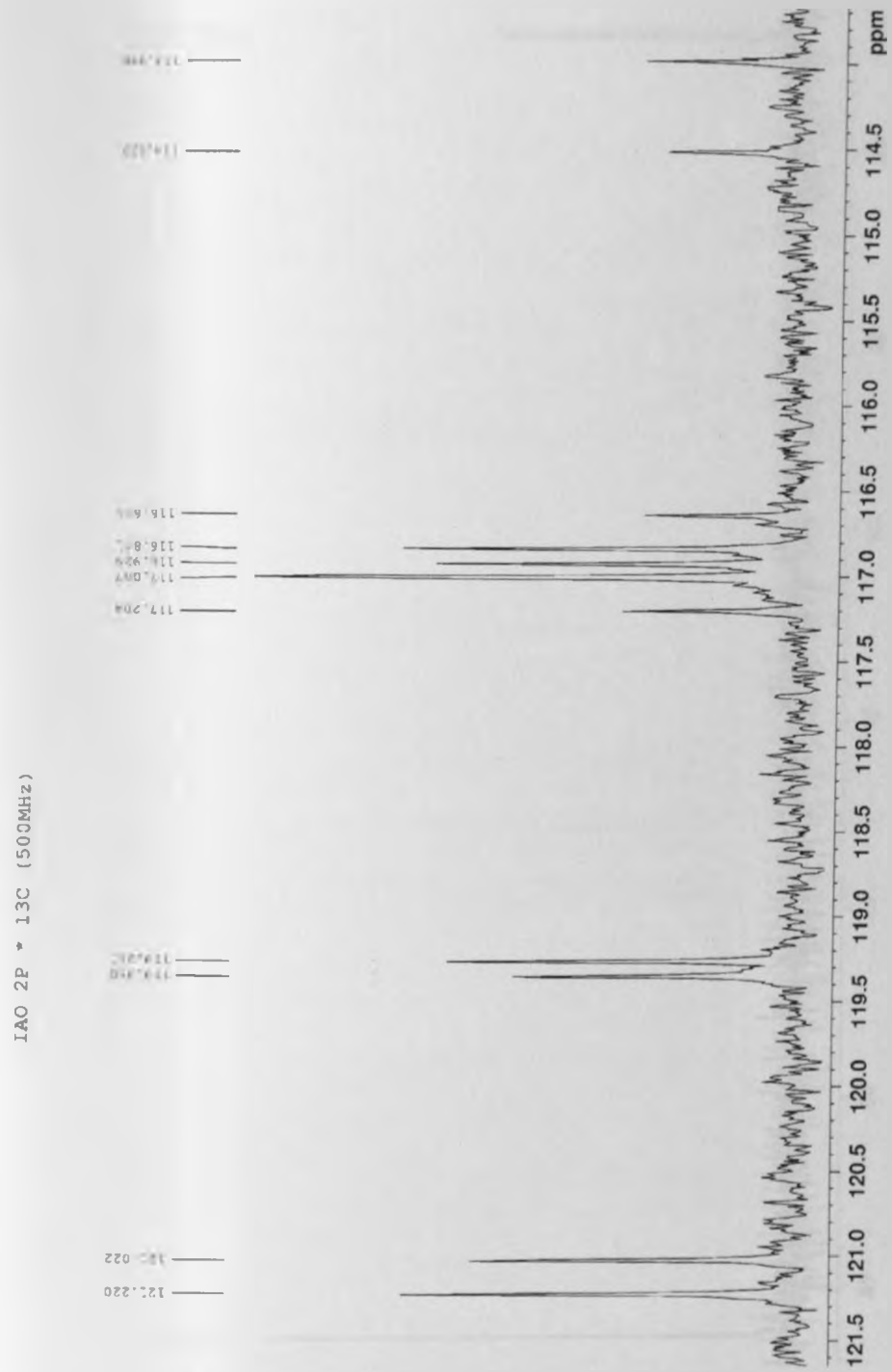
NMR SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125MHz)



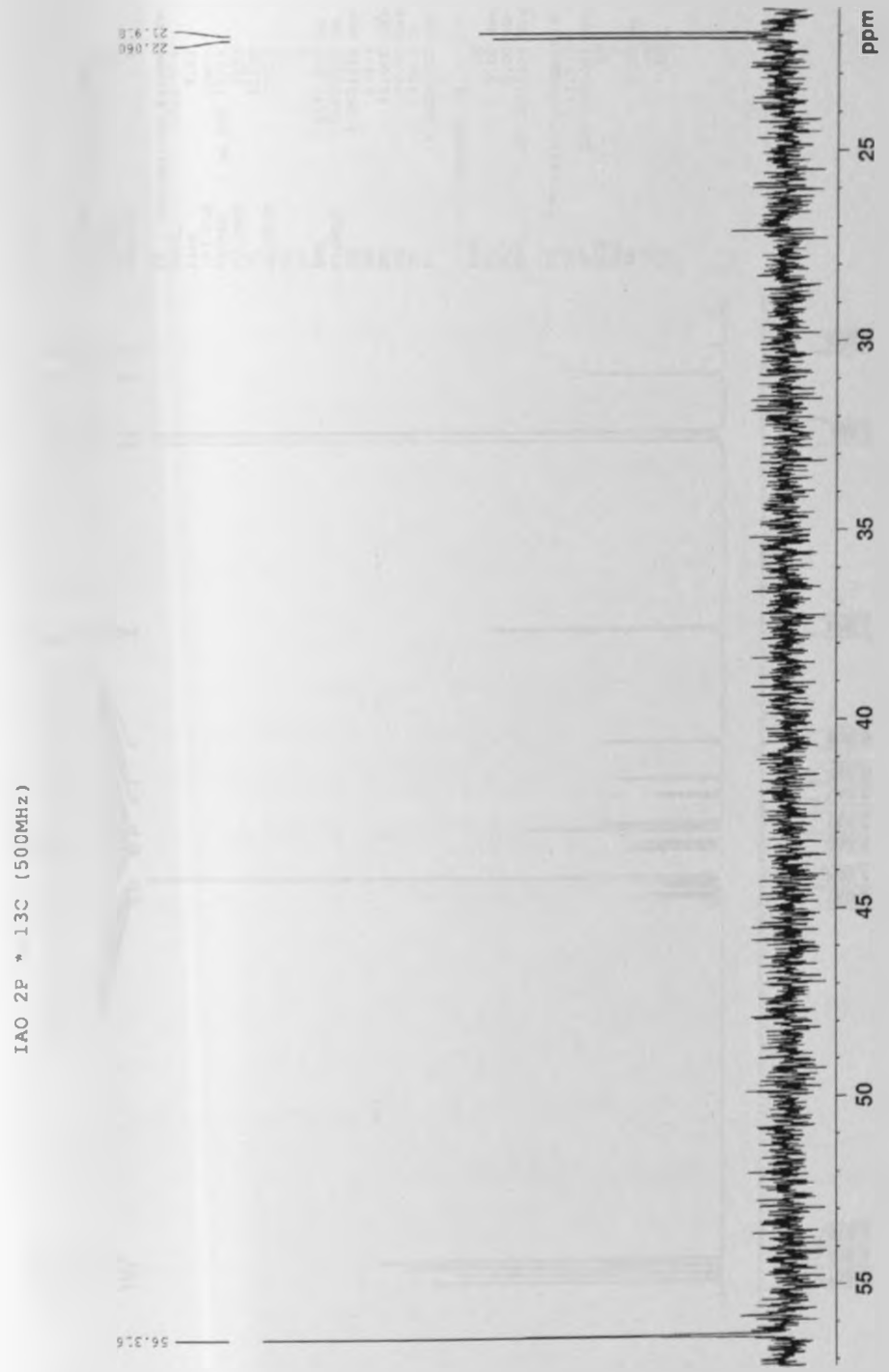
^{13}C NMR SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, ^{1}H-500 and ^{13}C-125MHz)



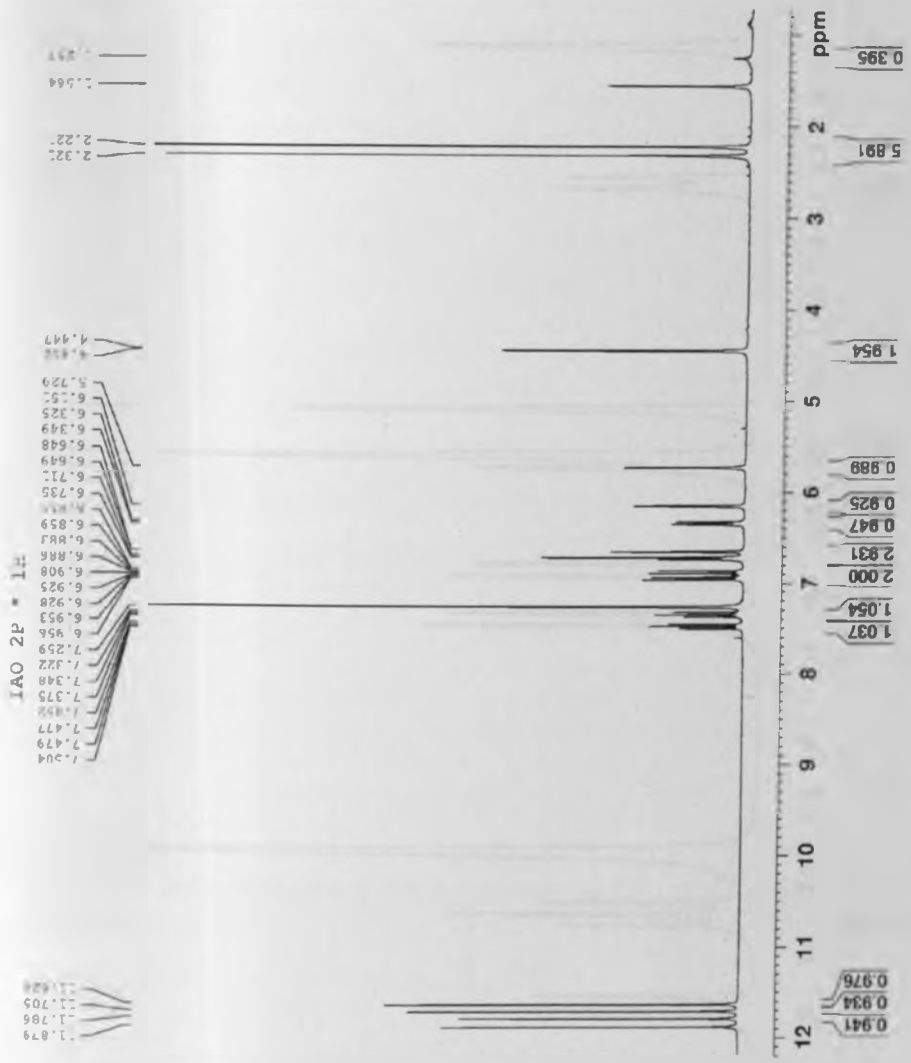
^{13}C NMR SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, ^{1}H-500 and ^{13}C-125MHz)



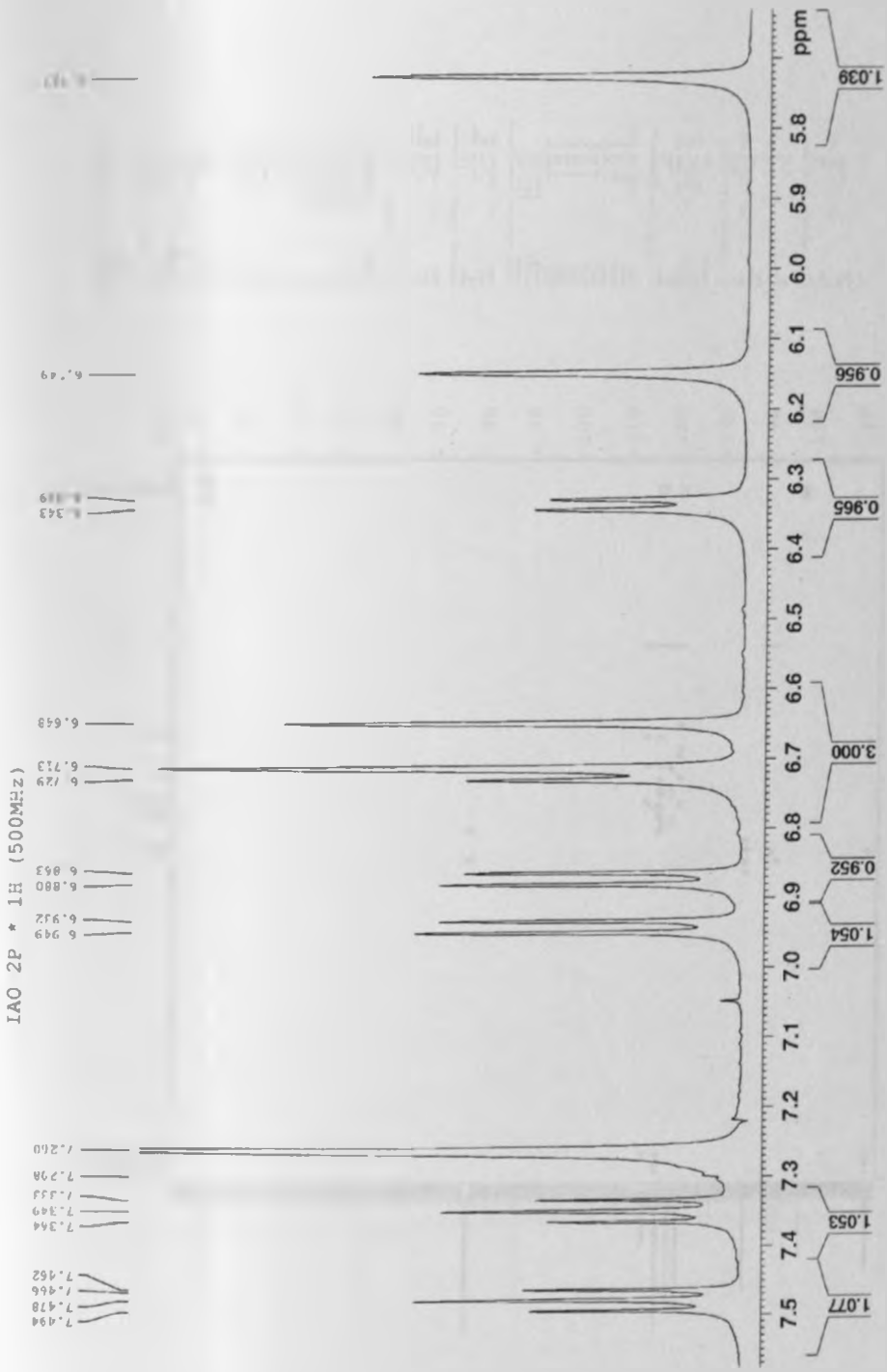
^{13}C NMR SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, ^{1}H-500 and ^{13}C-125MHz)



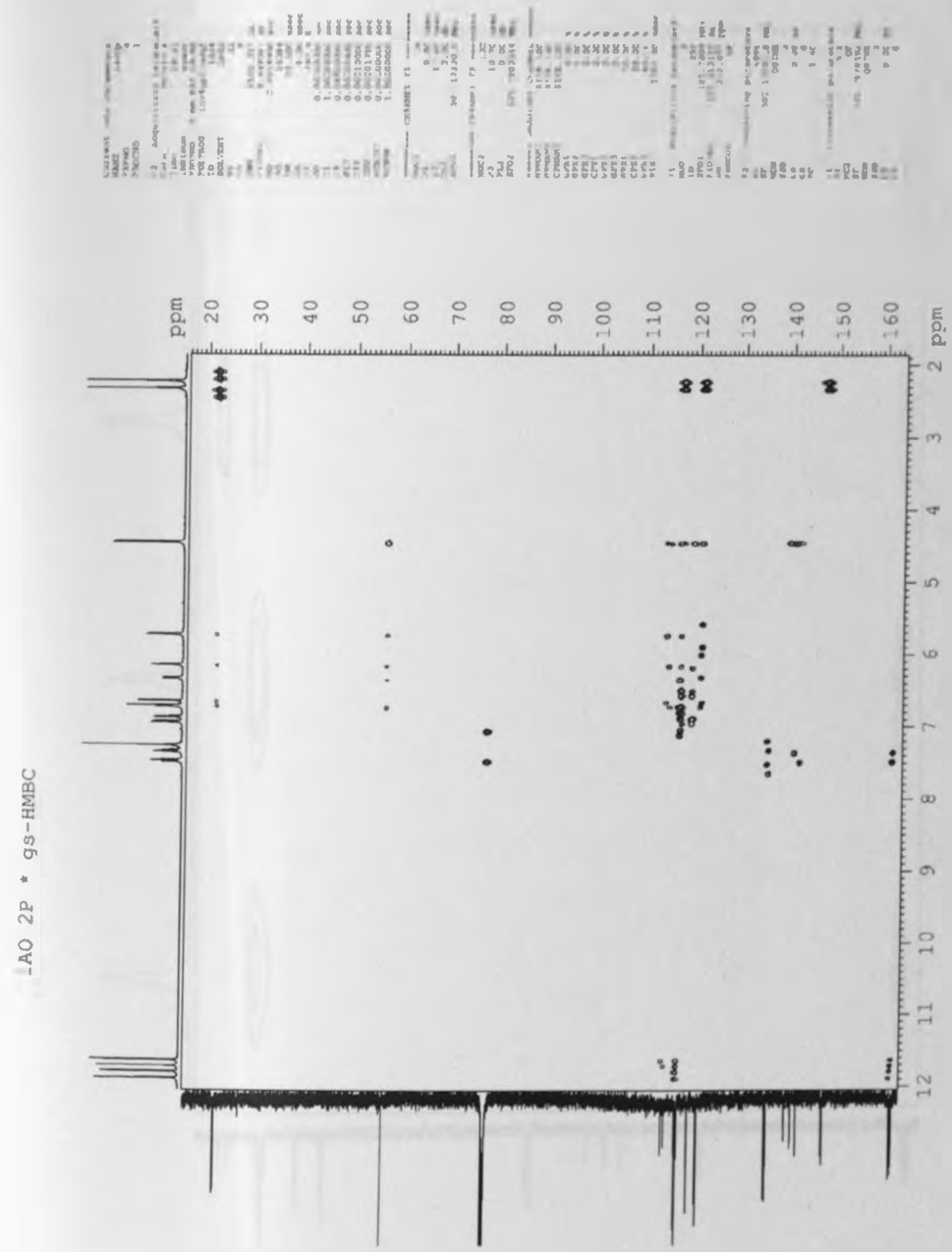
^{1}H NMR SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, 500 MHz)



^{1}H NMR SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, 500 MHz)

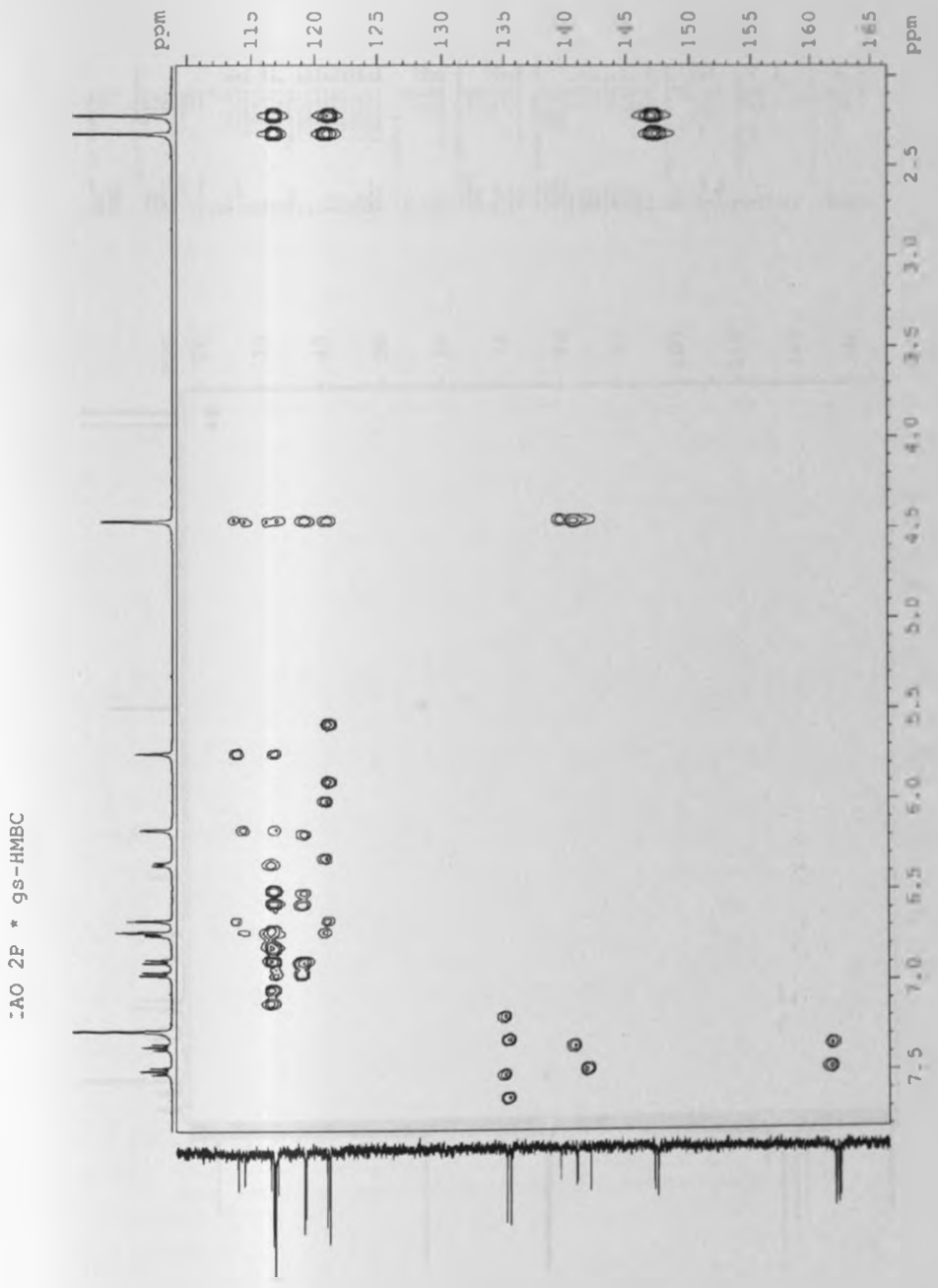


HMBC SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)

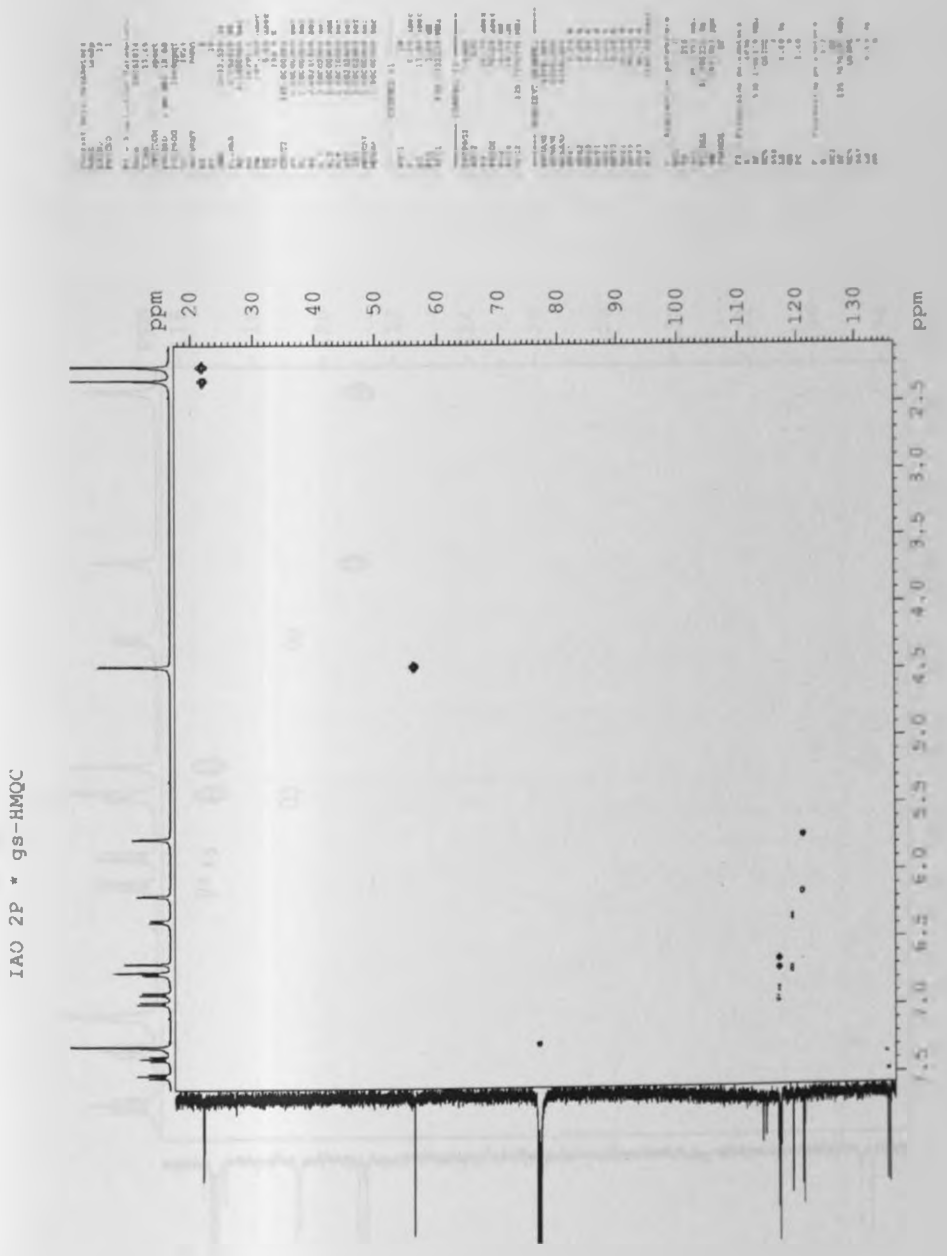


HMBC SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)

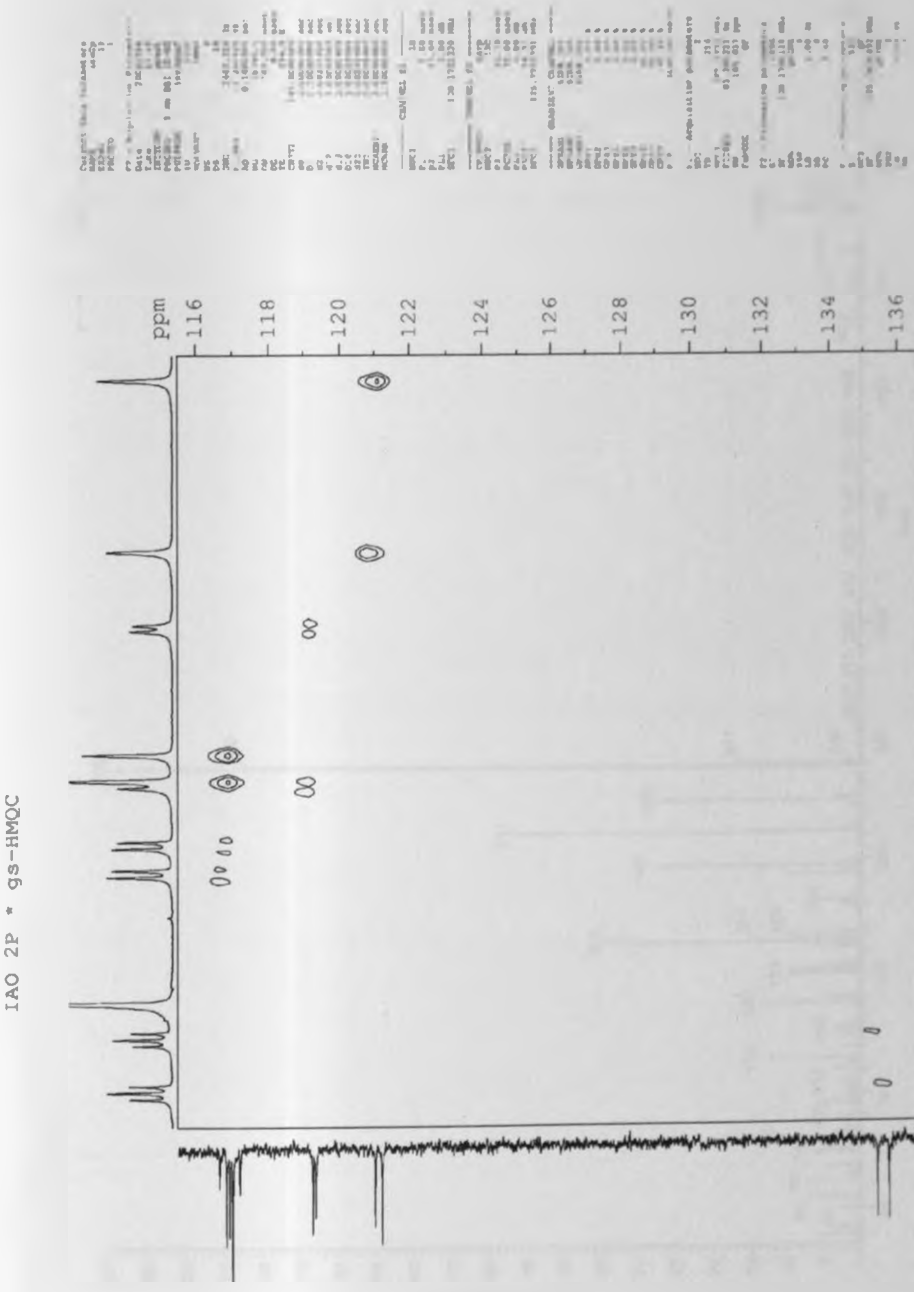
HMBC SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)



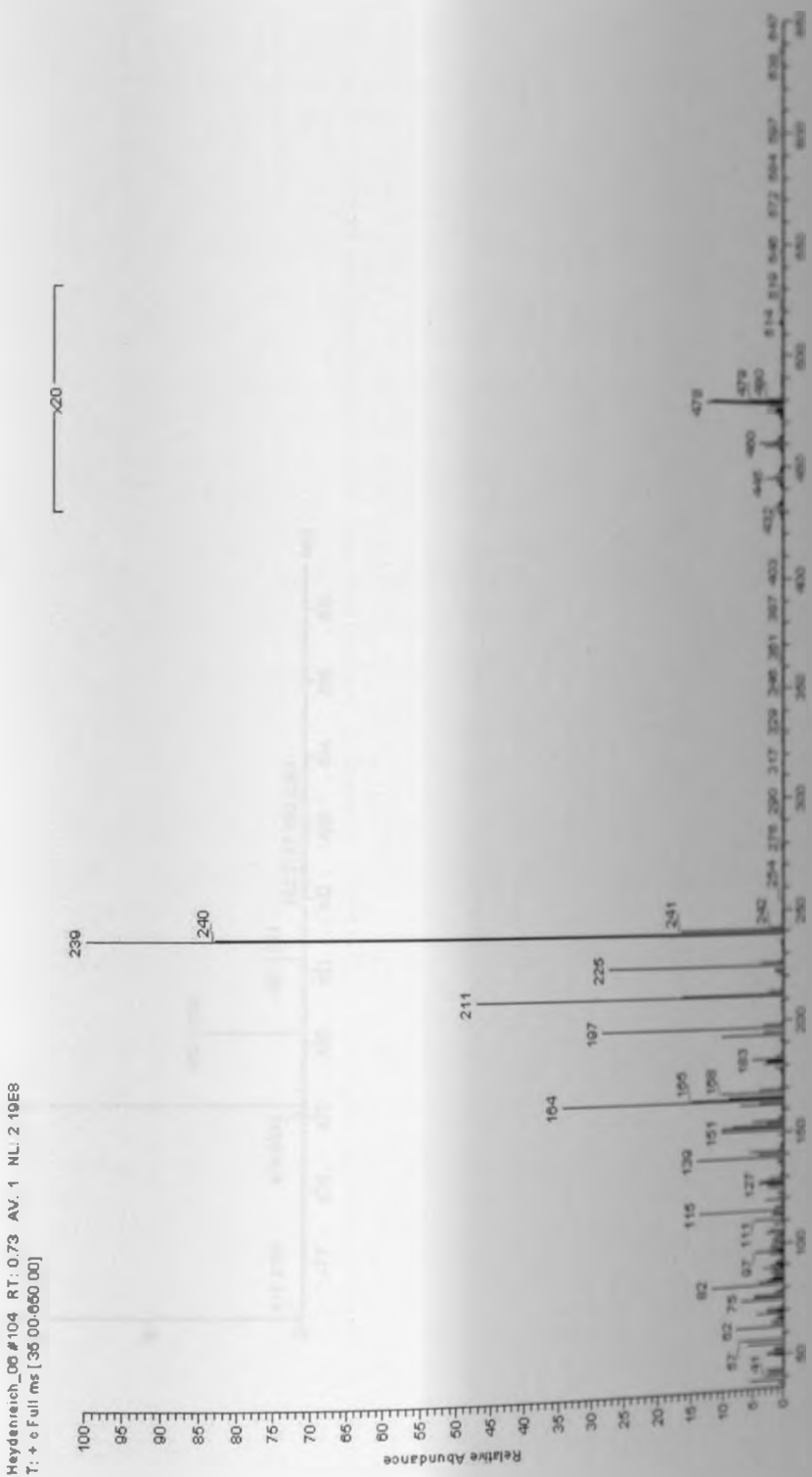
HMQC SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)



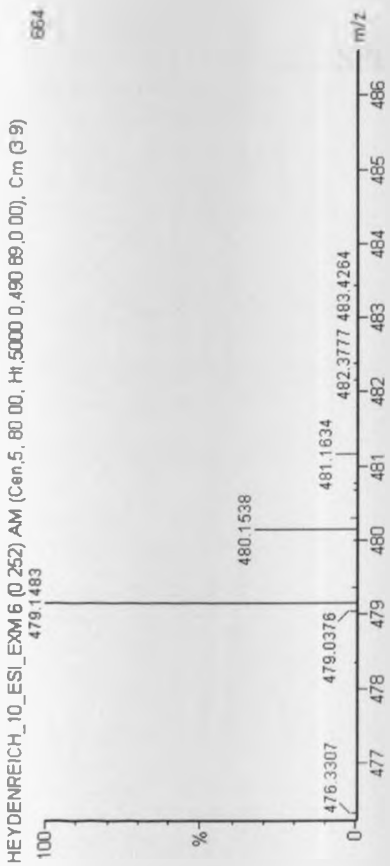
HMQC SPECTRUM FOR COMPOUND 9 (SOLVENT: $CDCl_3$, ^1H-500 and ^{13}C-125 MHz)



MS FOR COMPOUND 9

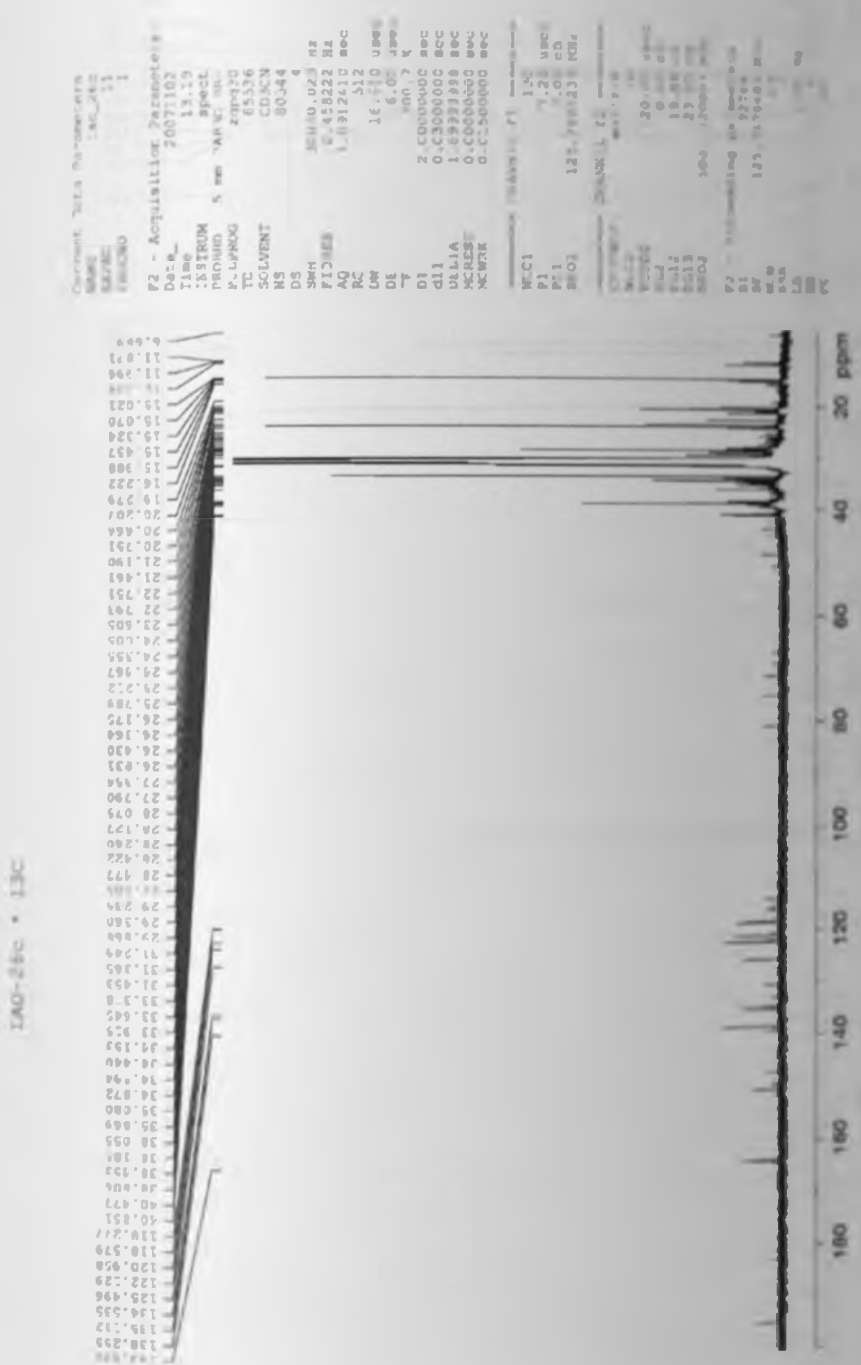


HRMS FOR COMPOUND 9

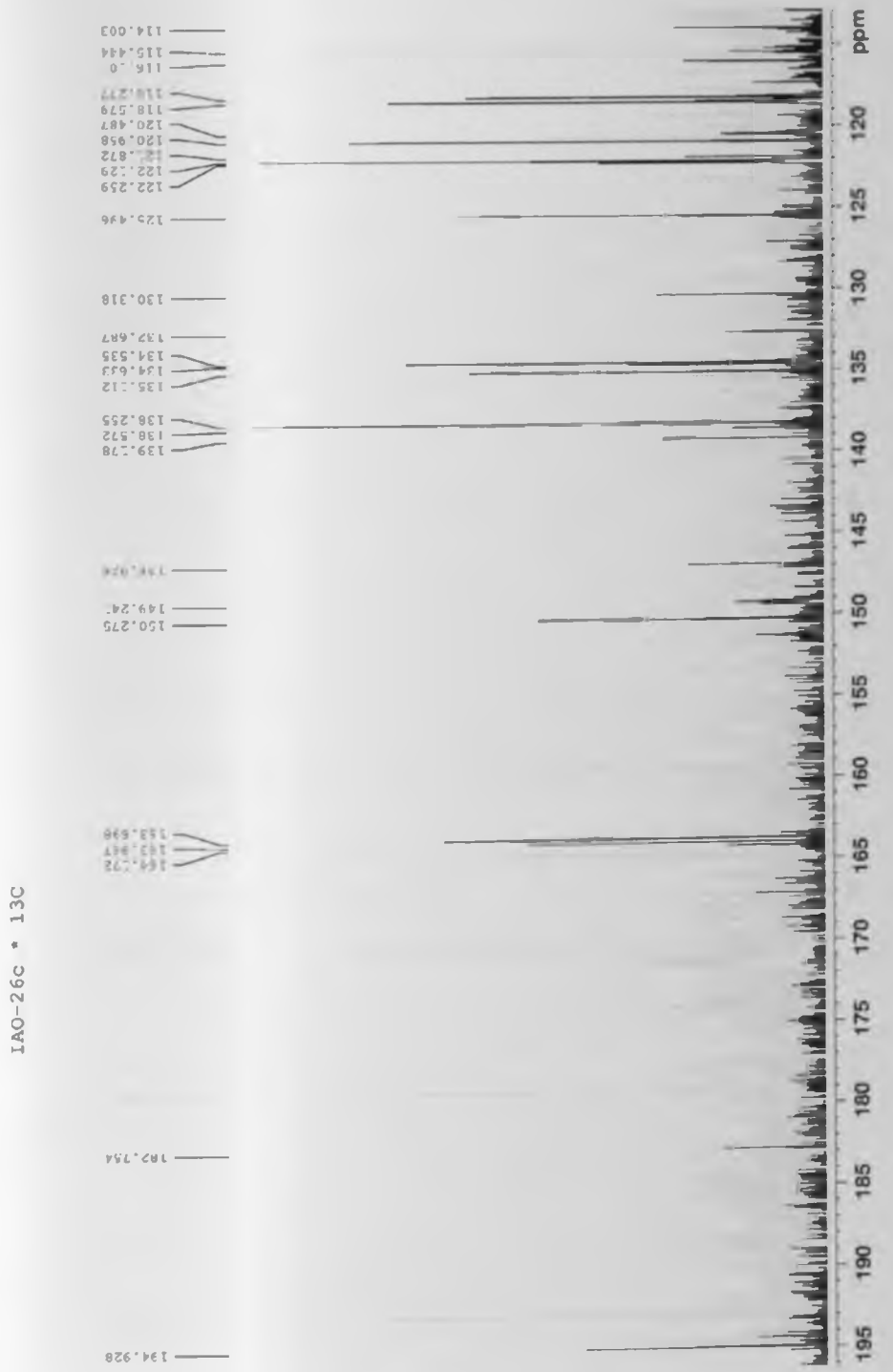


SPECTRA FOR COMPOUND 10

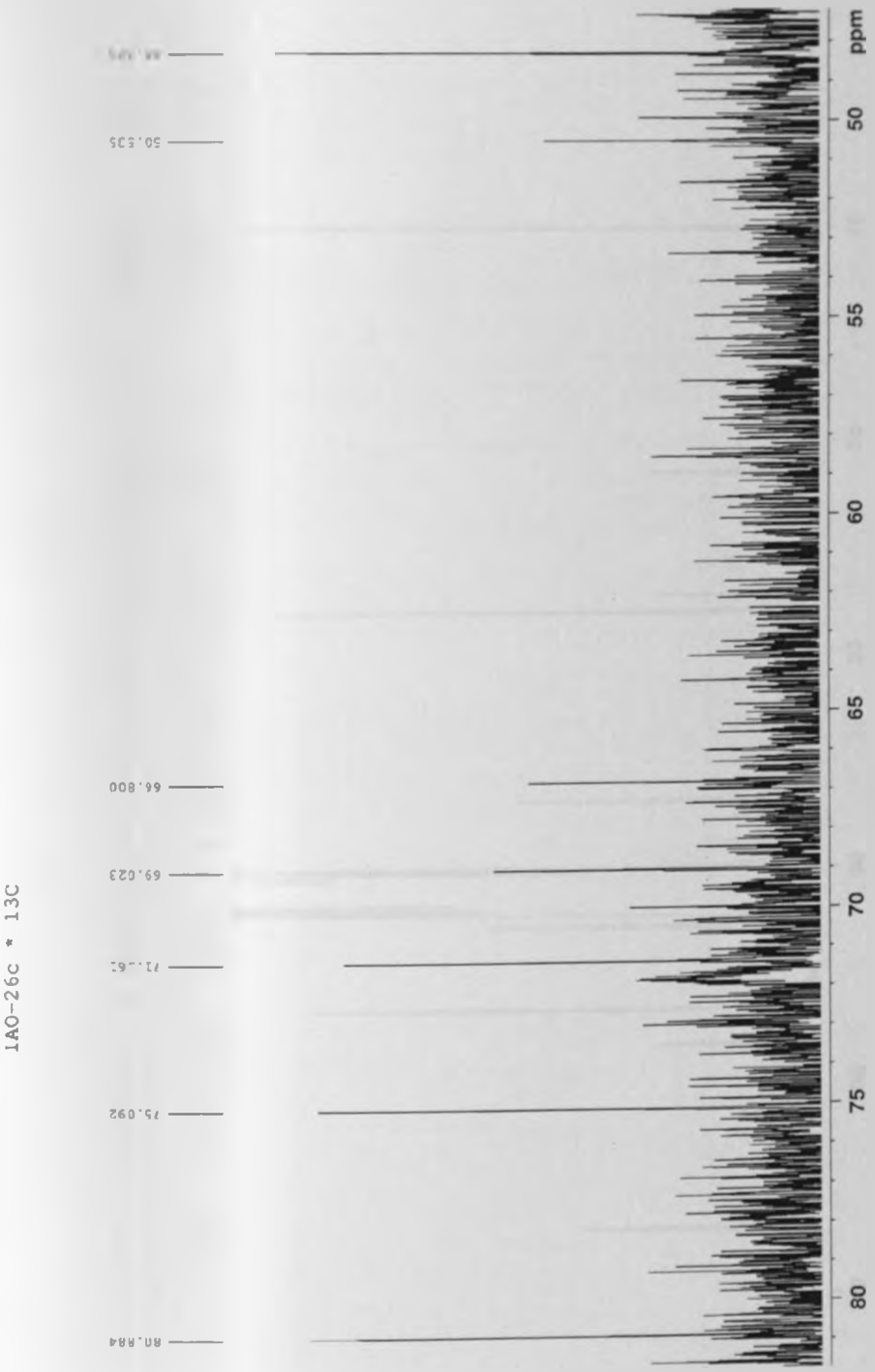
^{13}C NMR SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, 125 MHz)



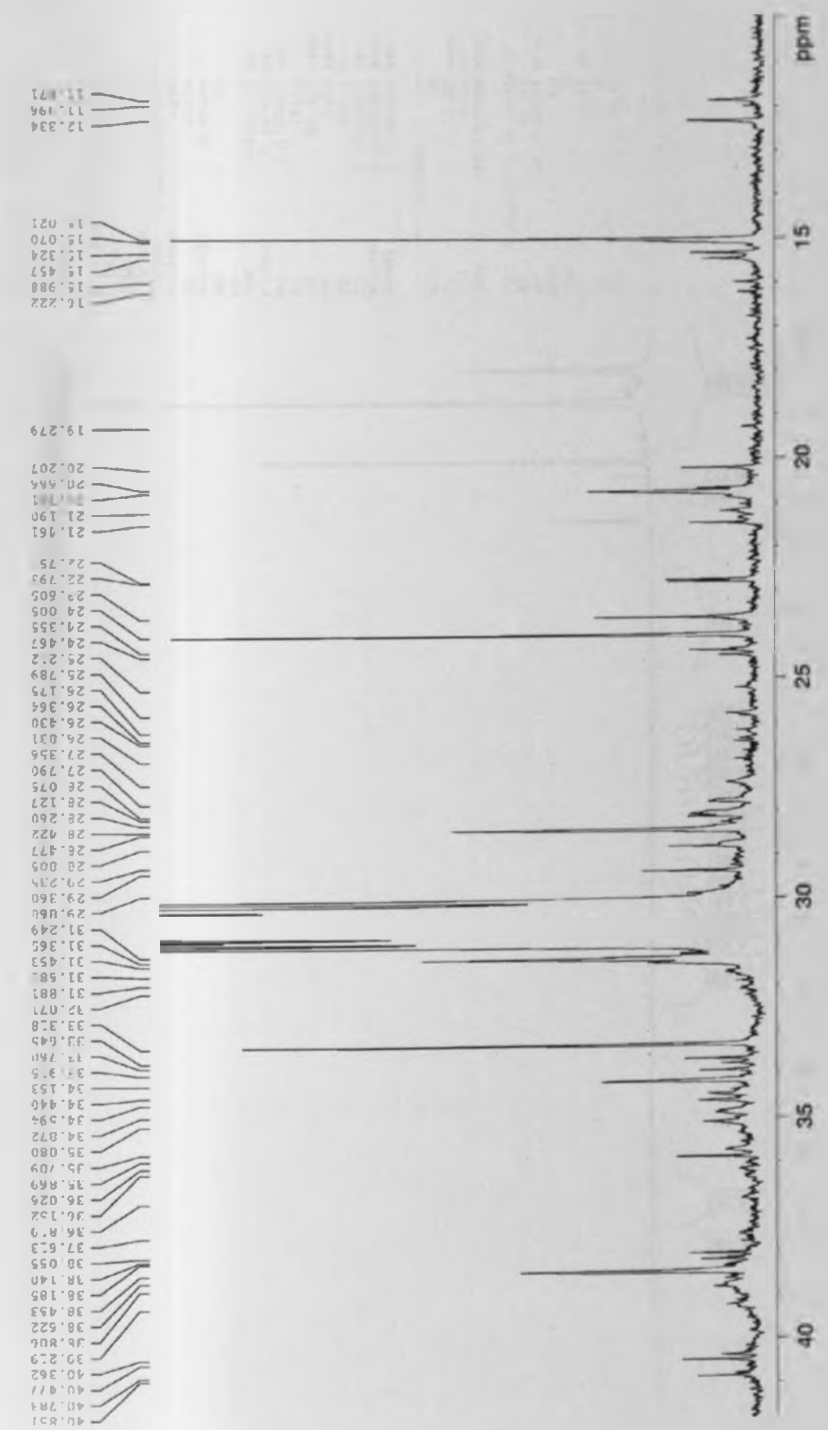
^{13}C NMR SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, 125 MHz)



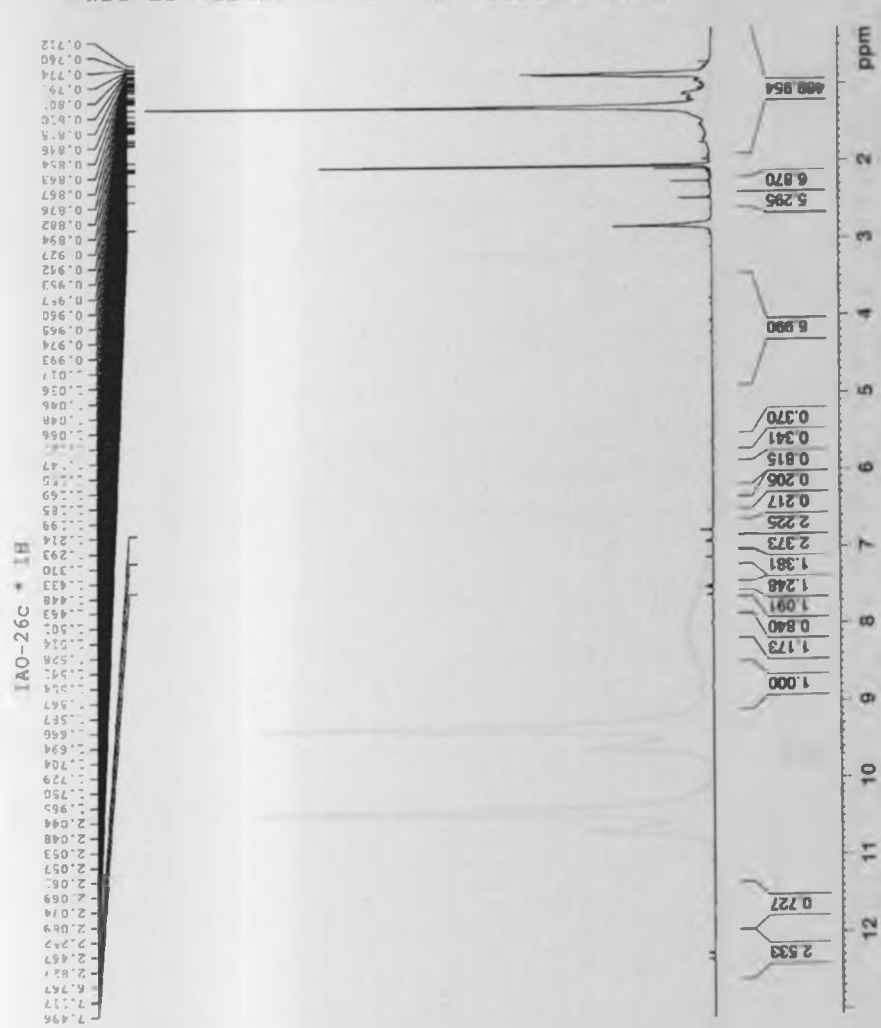
^{13}C NMR SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, 125 MHz)



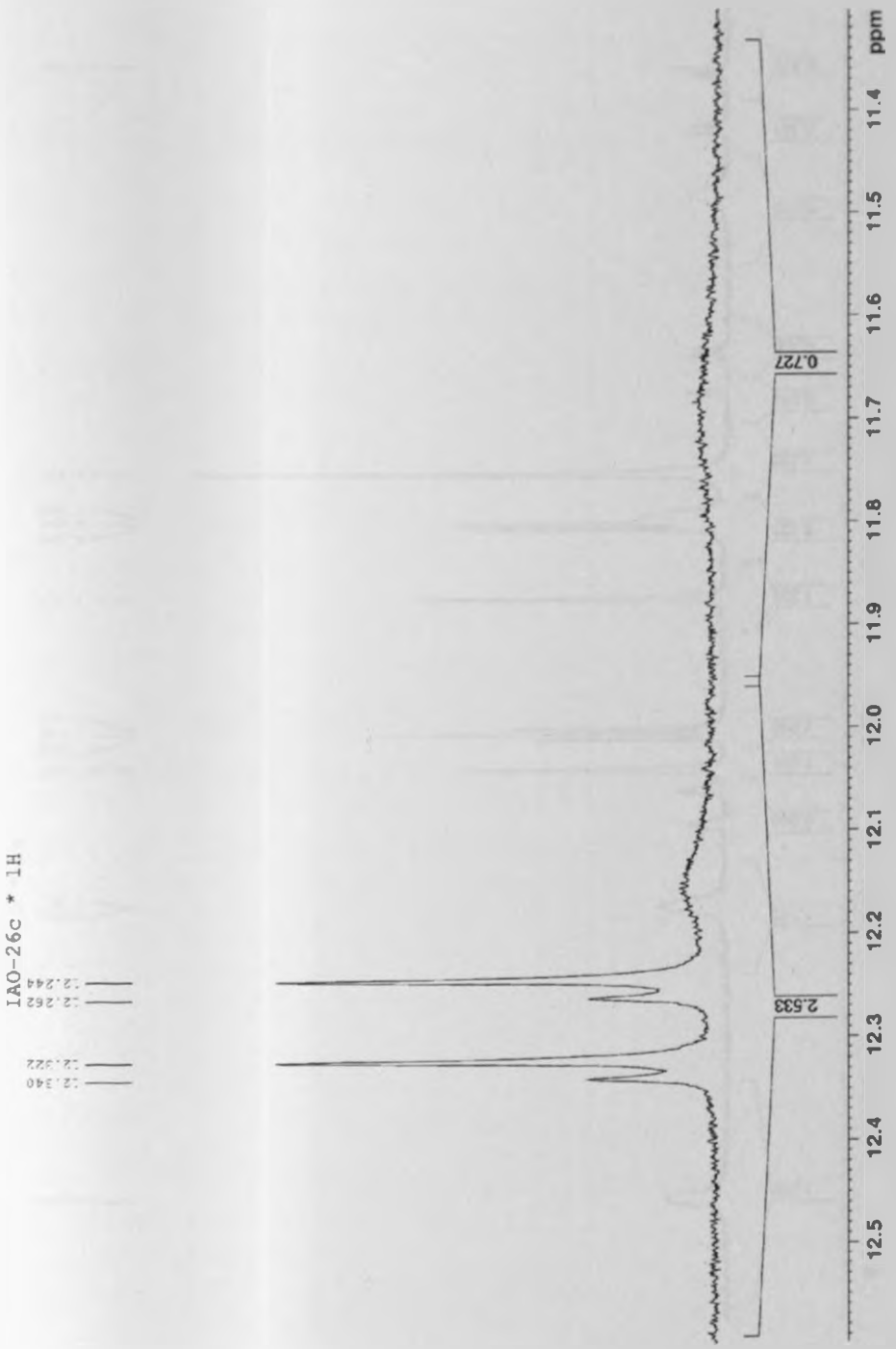
^{13}C NMR SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, 125 MHz)



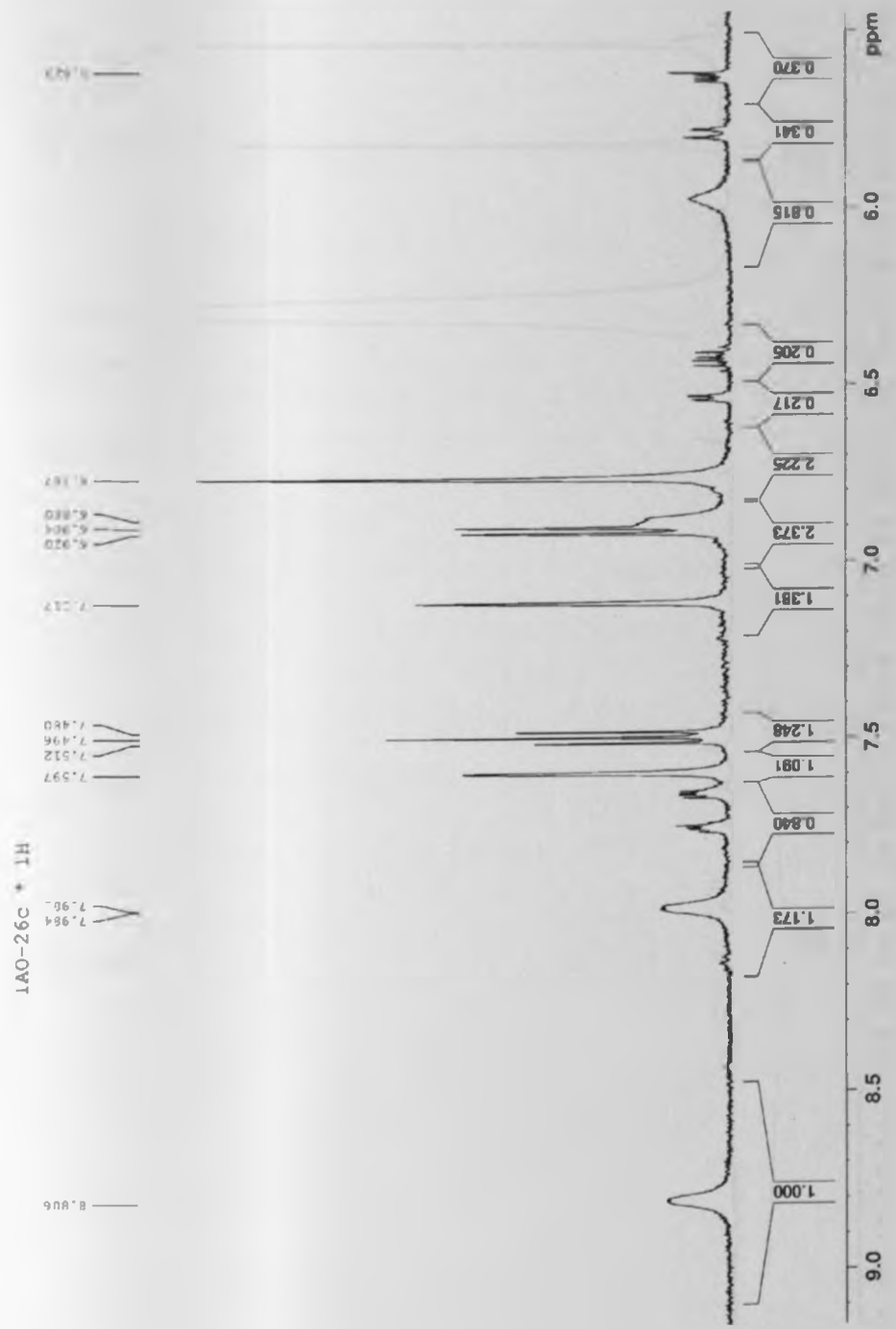
^{1}H NMR SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, 500 MHz)



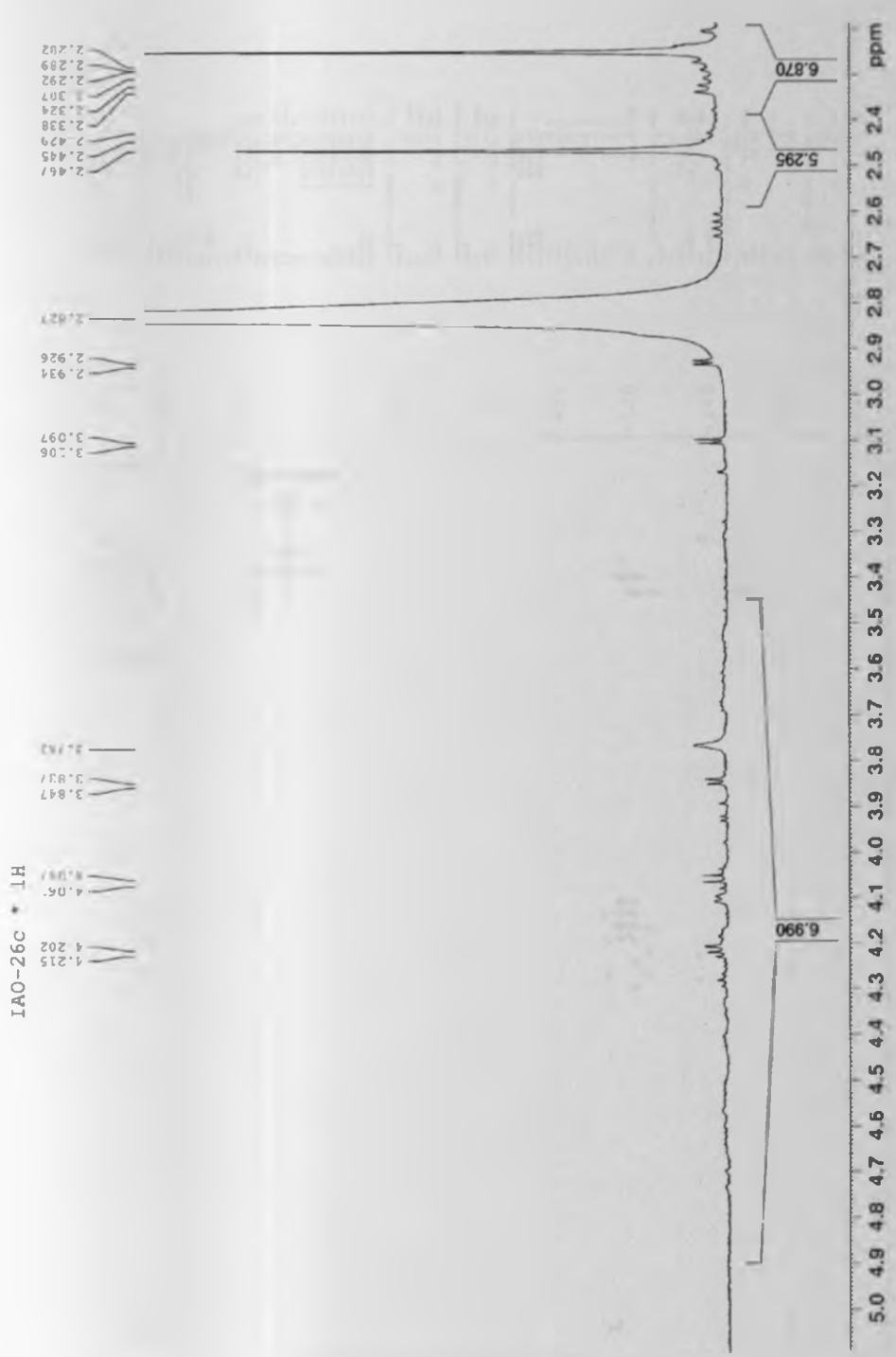
^{1}H NMR SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, 500 MHz)



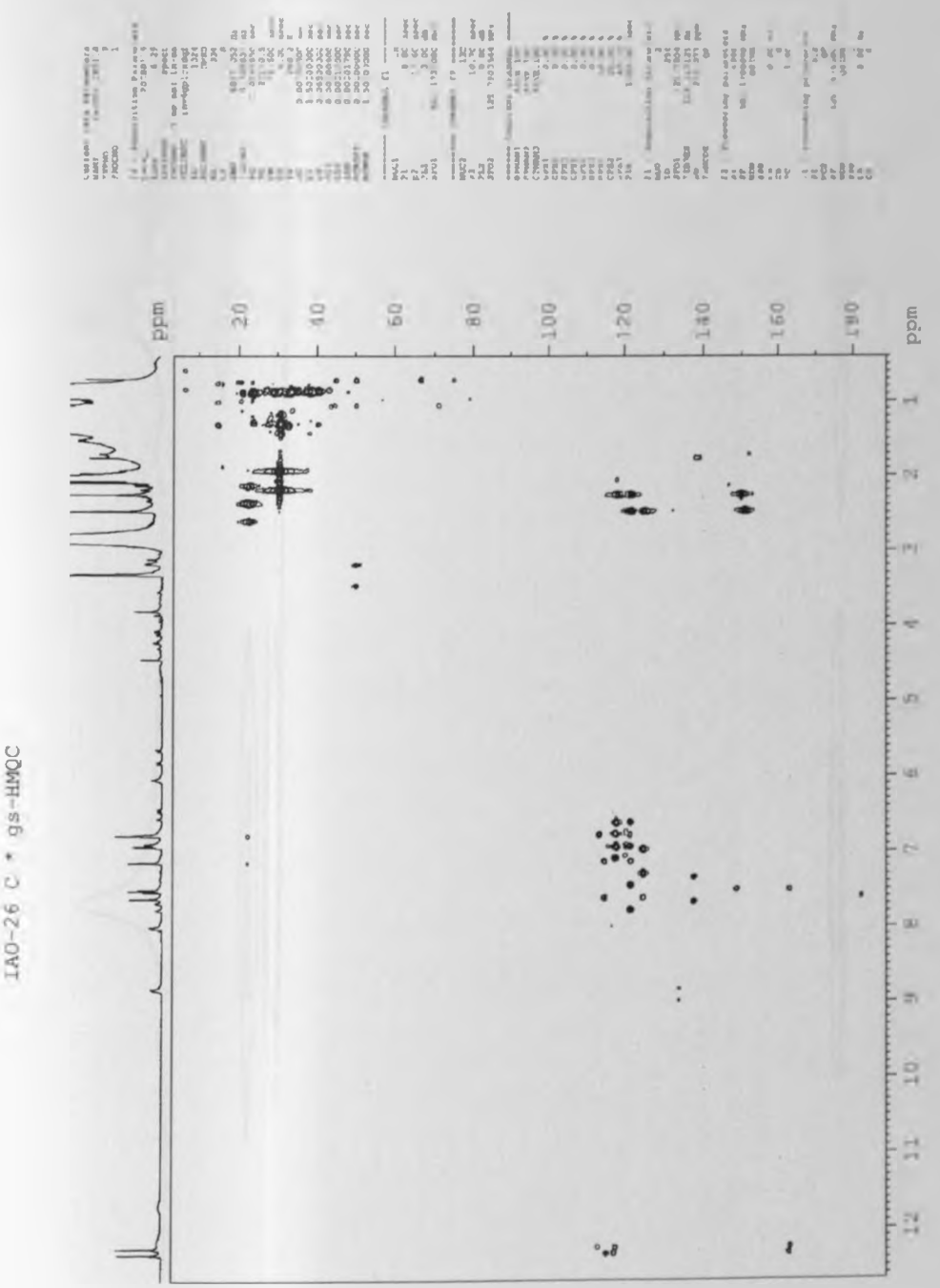
^{1}H NMR SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, 500 MHz)



^{1}H NMR SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, 500 MHz)

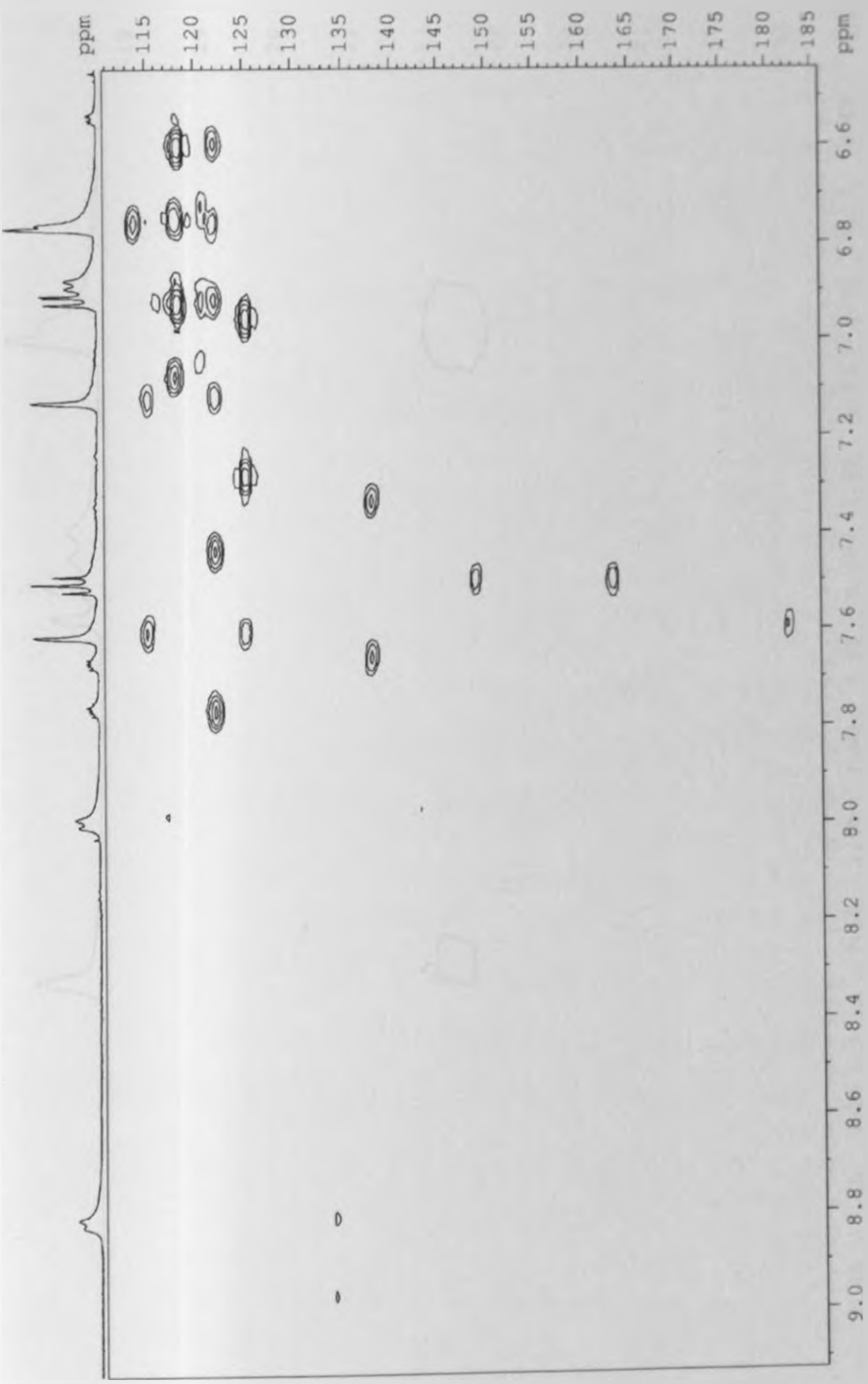


HMBC SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125MHz)



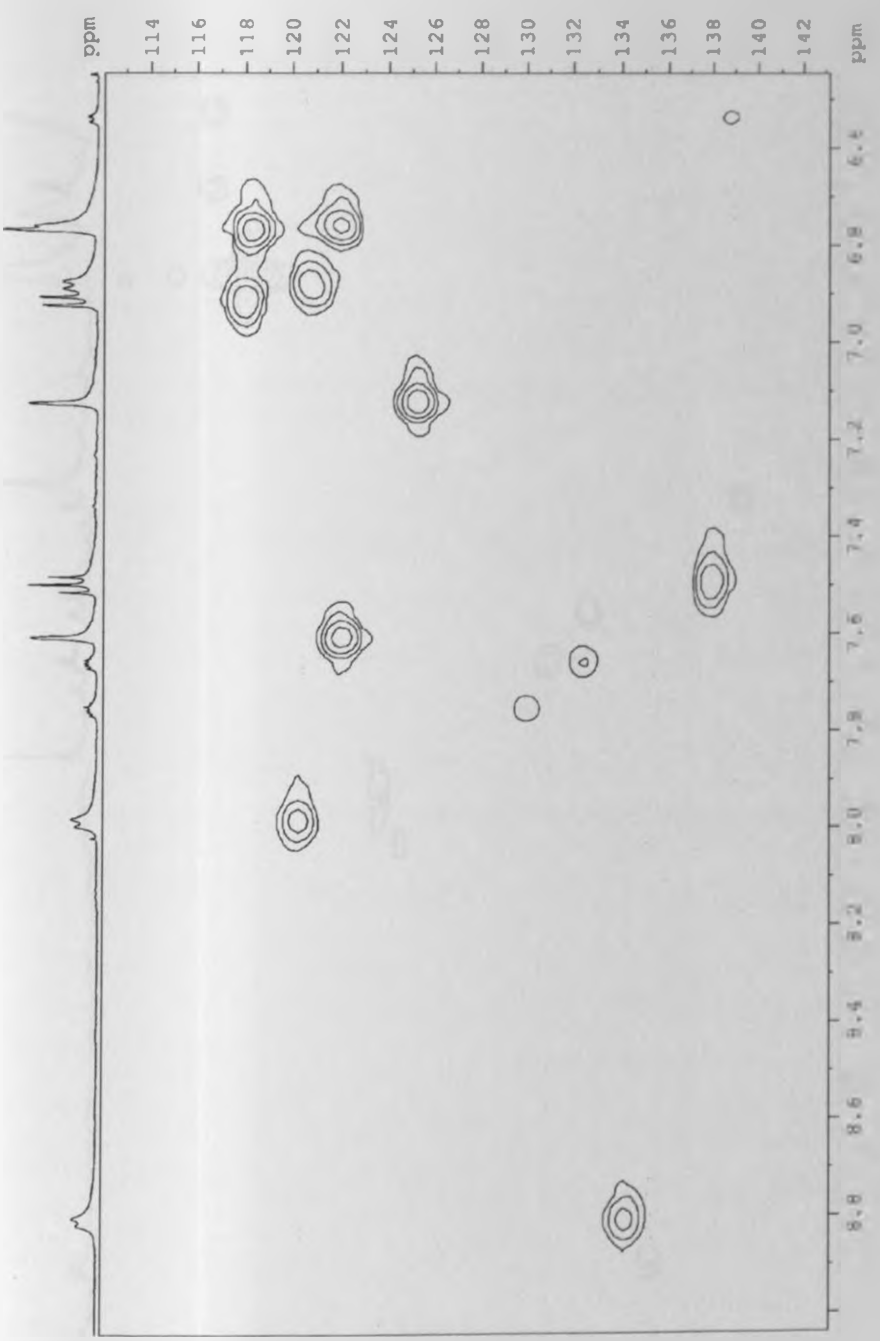
HMBC SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125MHz)

HMBC SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125MHz)



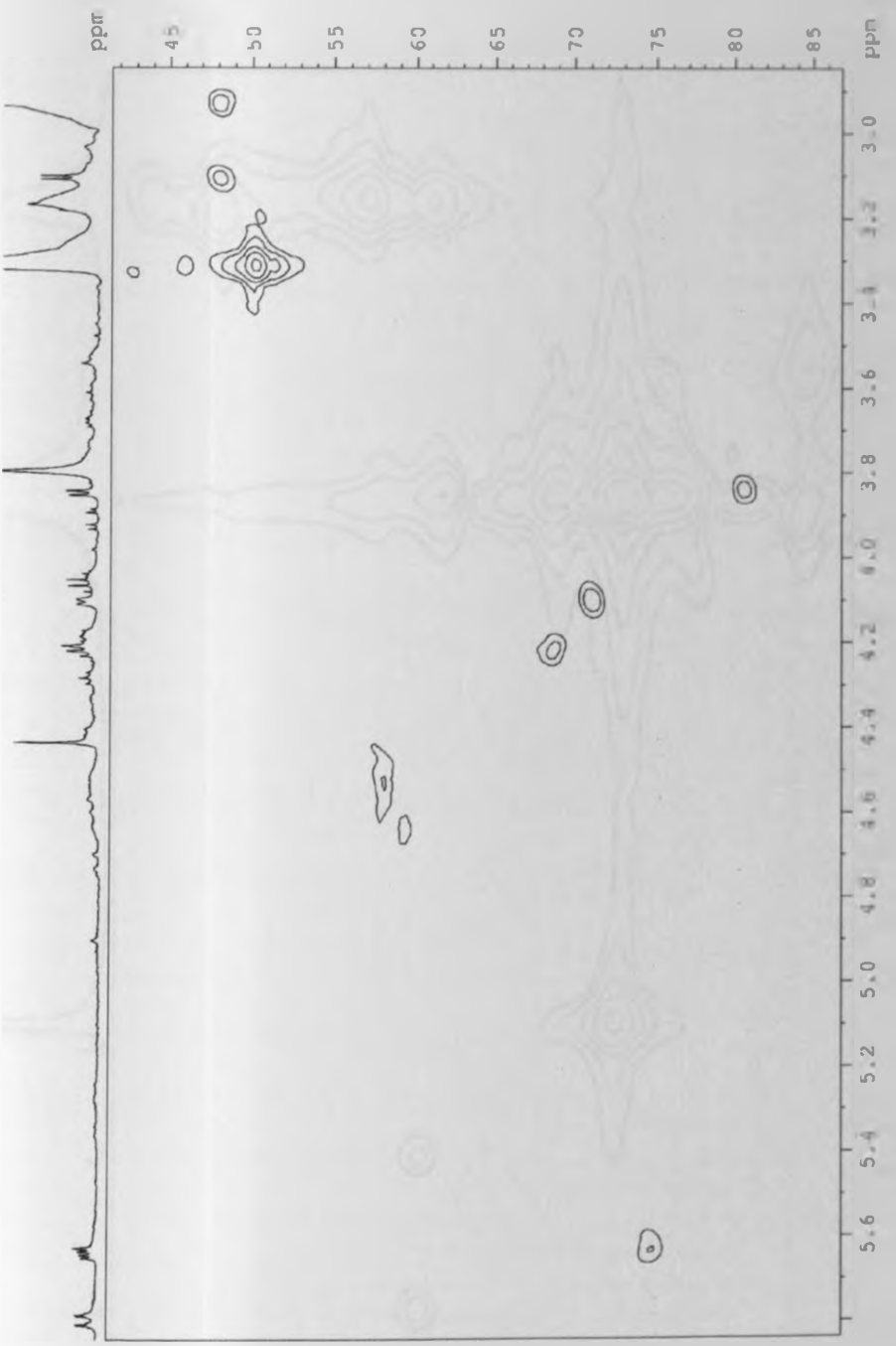
HMBC SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125MHz)

HMQC SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125MHz)



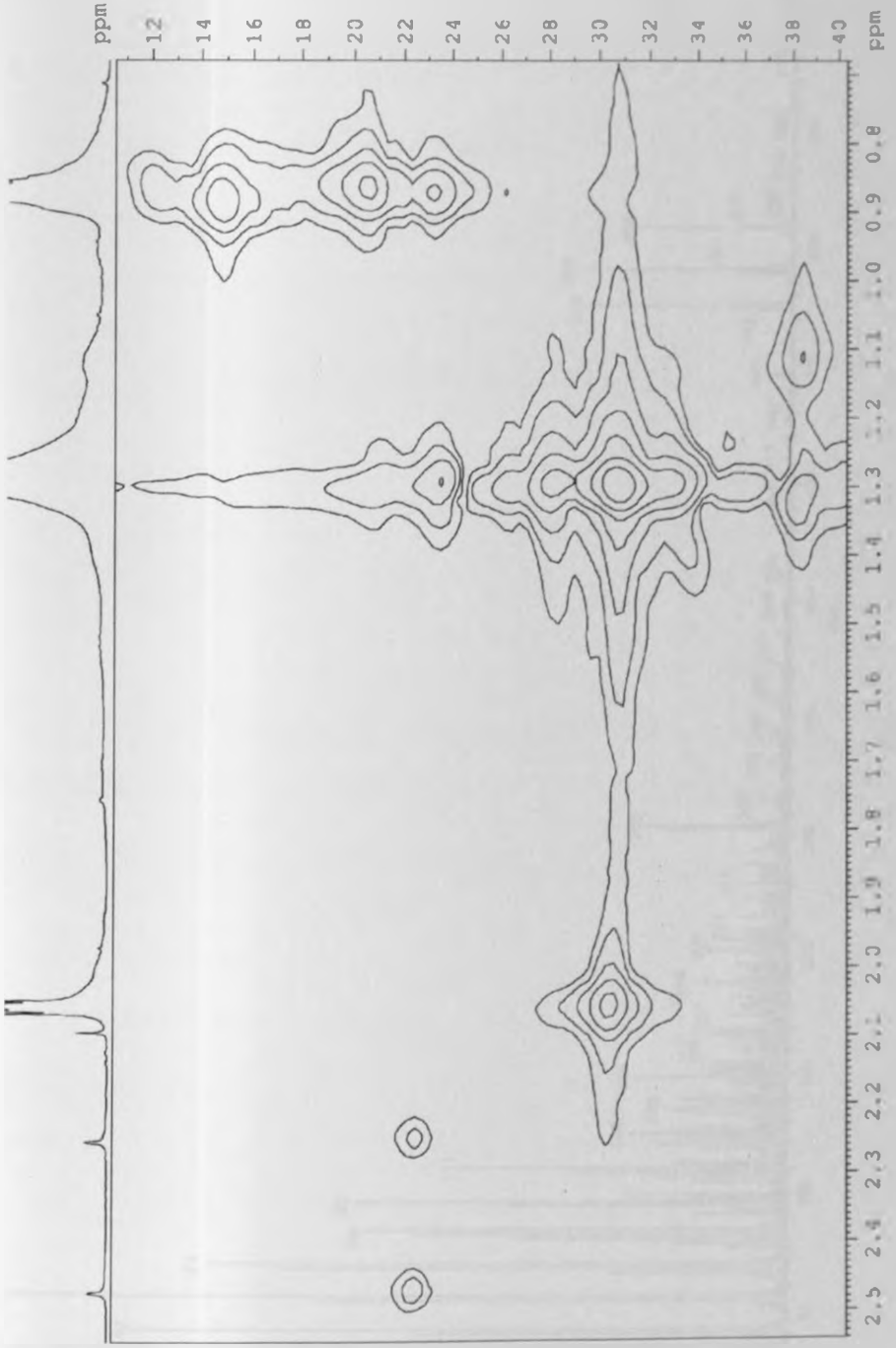
AO-26 C * gs-HMQC

HMQC SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125MHz)

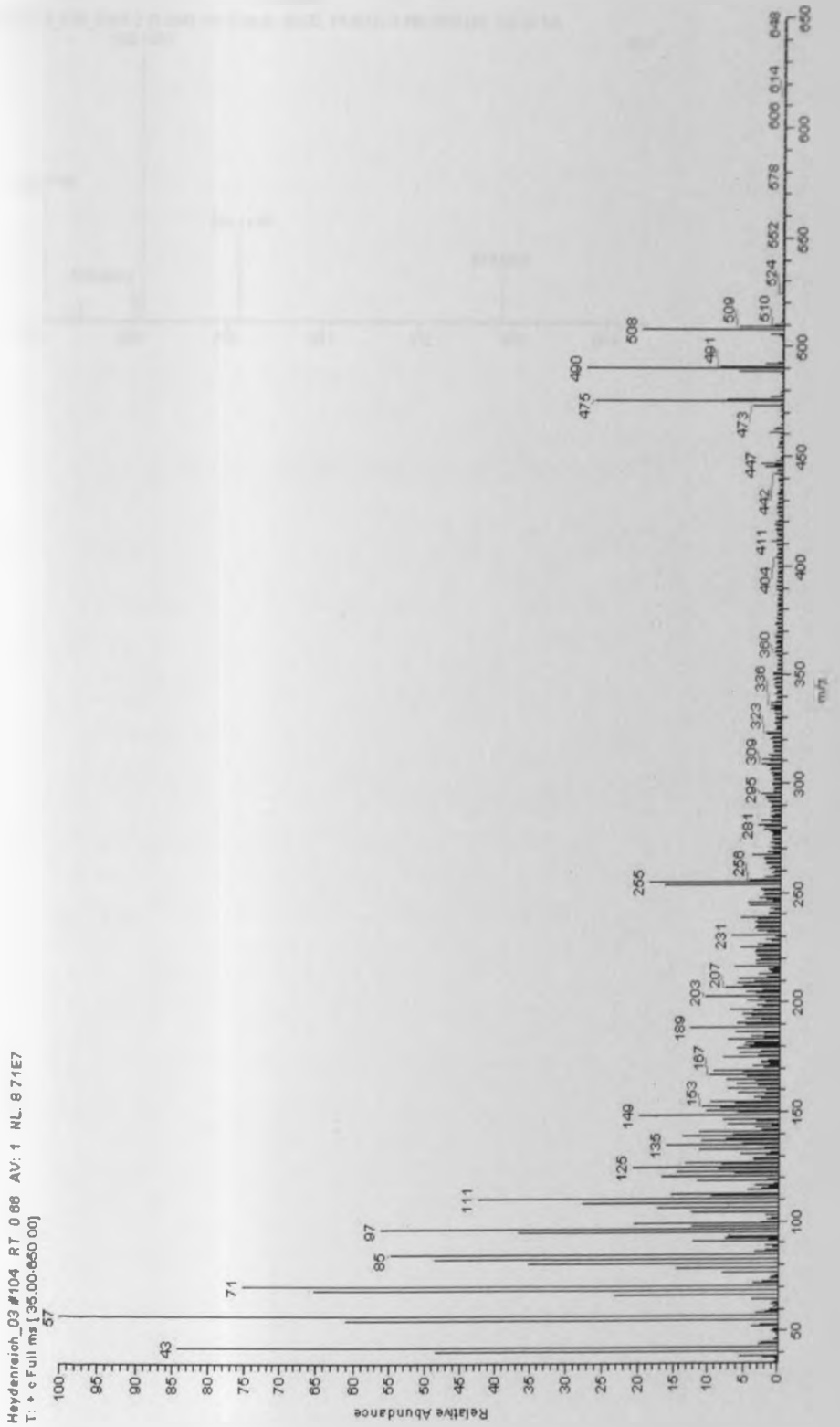


1AO-26 C * gs-HMQC

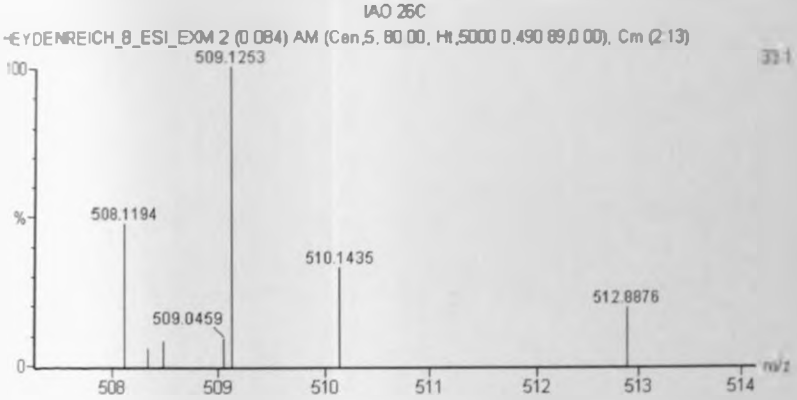
HMQC SPECTRUM FOR COMPOUND 10 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125MHz)



MS FOR COMPOUND 10

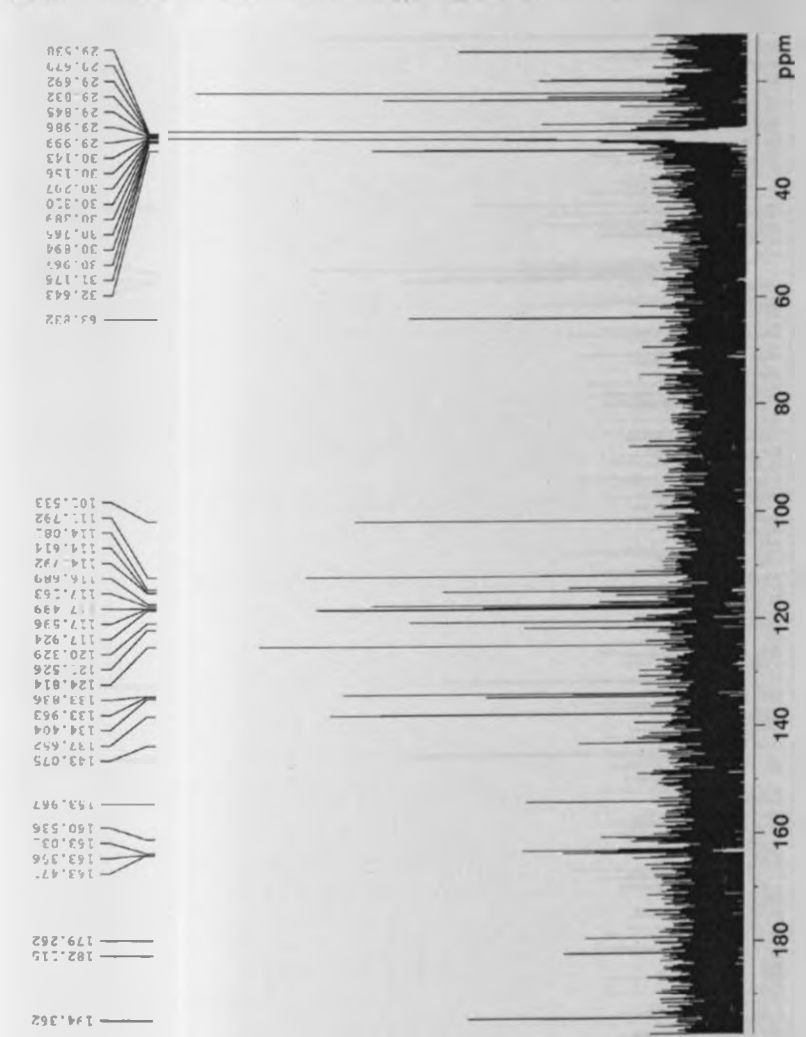


HRMS FOR COMPOUND 10

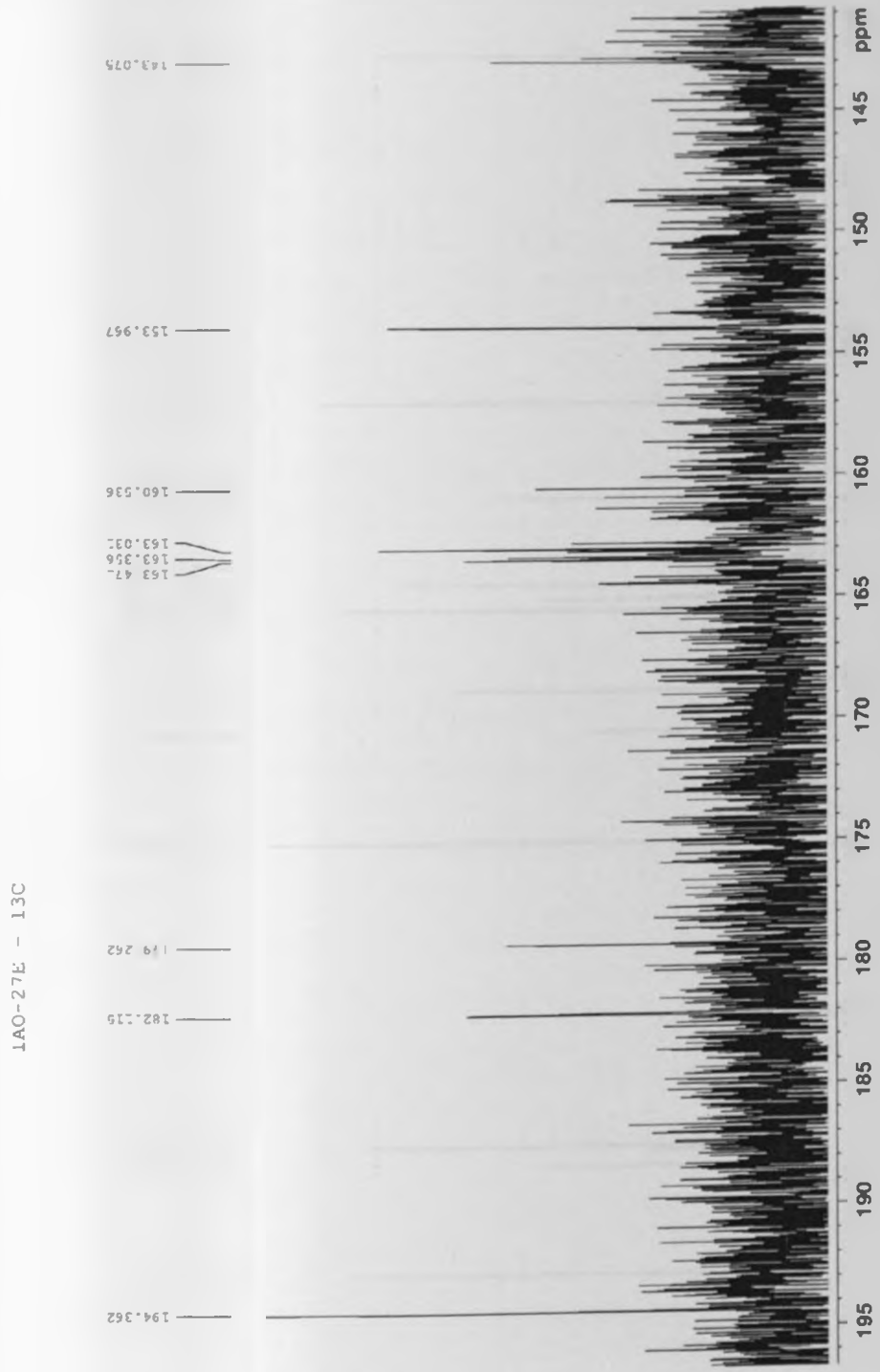


SPECTRA FOR COMPOUND 11

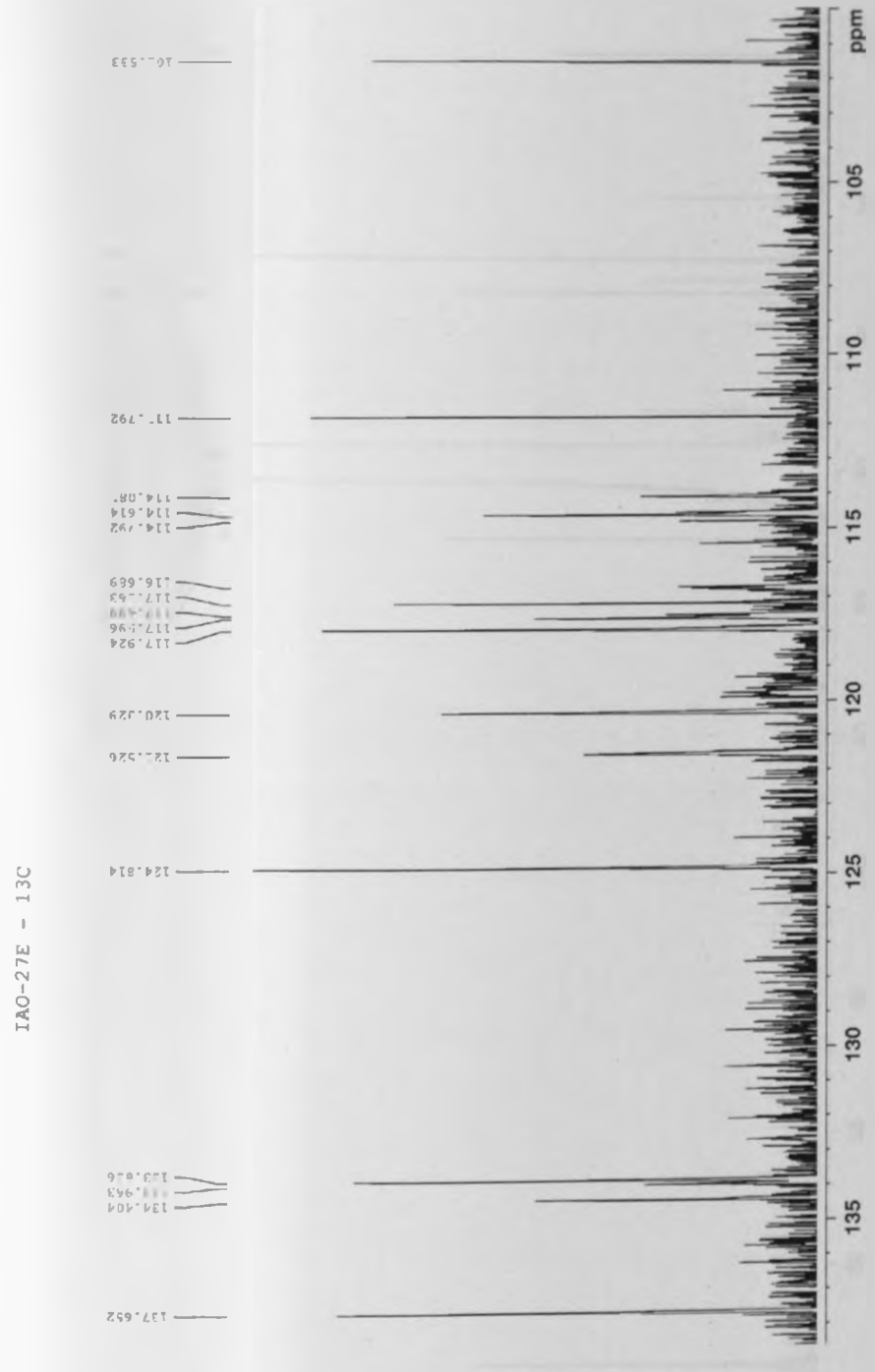
^{13}C NMR SPECTRUM FOR COMPOUND 11 (SOLVENT: ACETONE-d_6, 125 MHz)



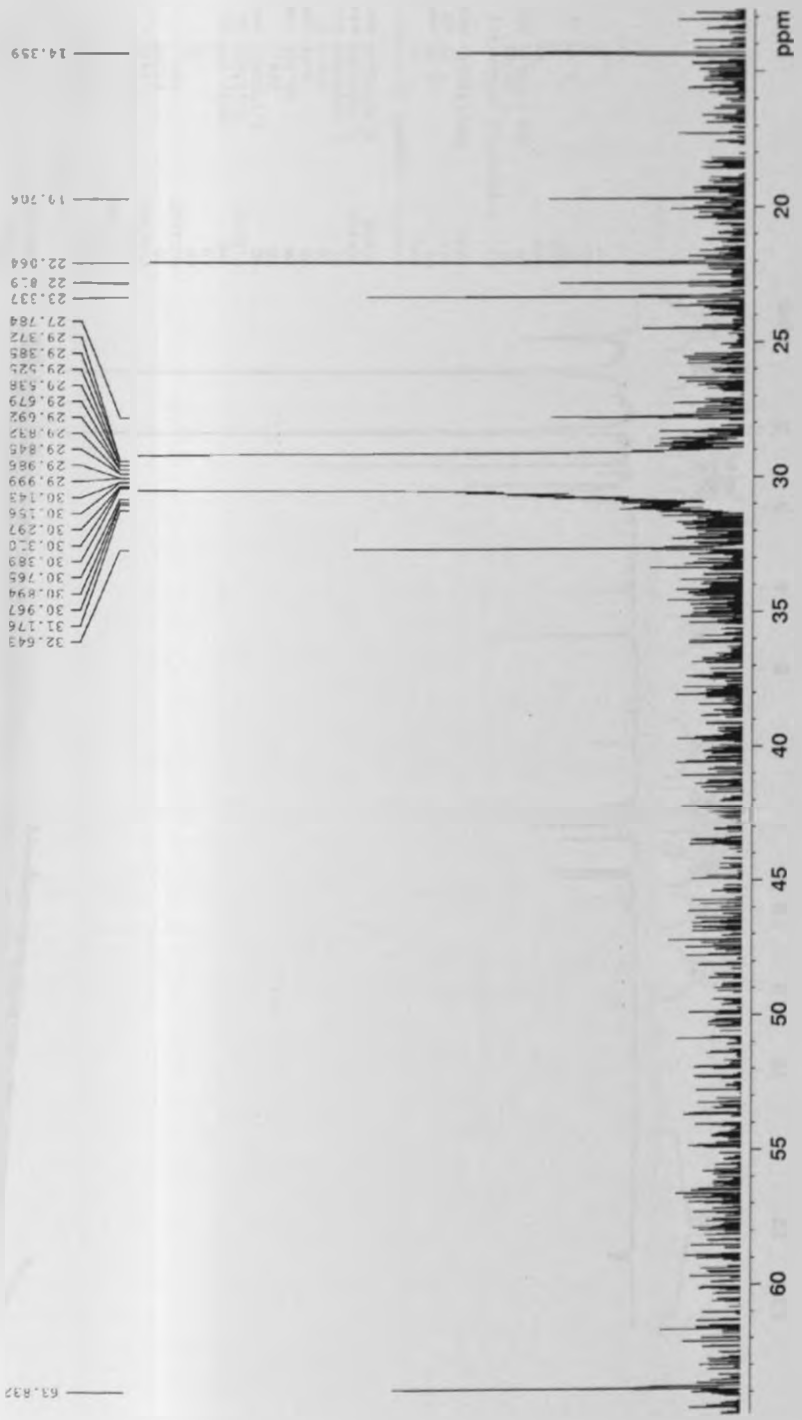
^{13}C NMR SPECTRUM FOR COMPOUND 11 (SOLVENT: ACETONE-d_6, 125 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND 11 (SOLVENT: ACETONE-d_6, 125 MHz)

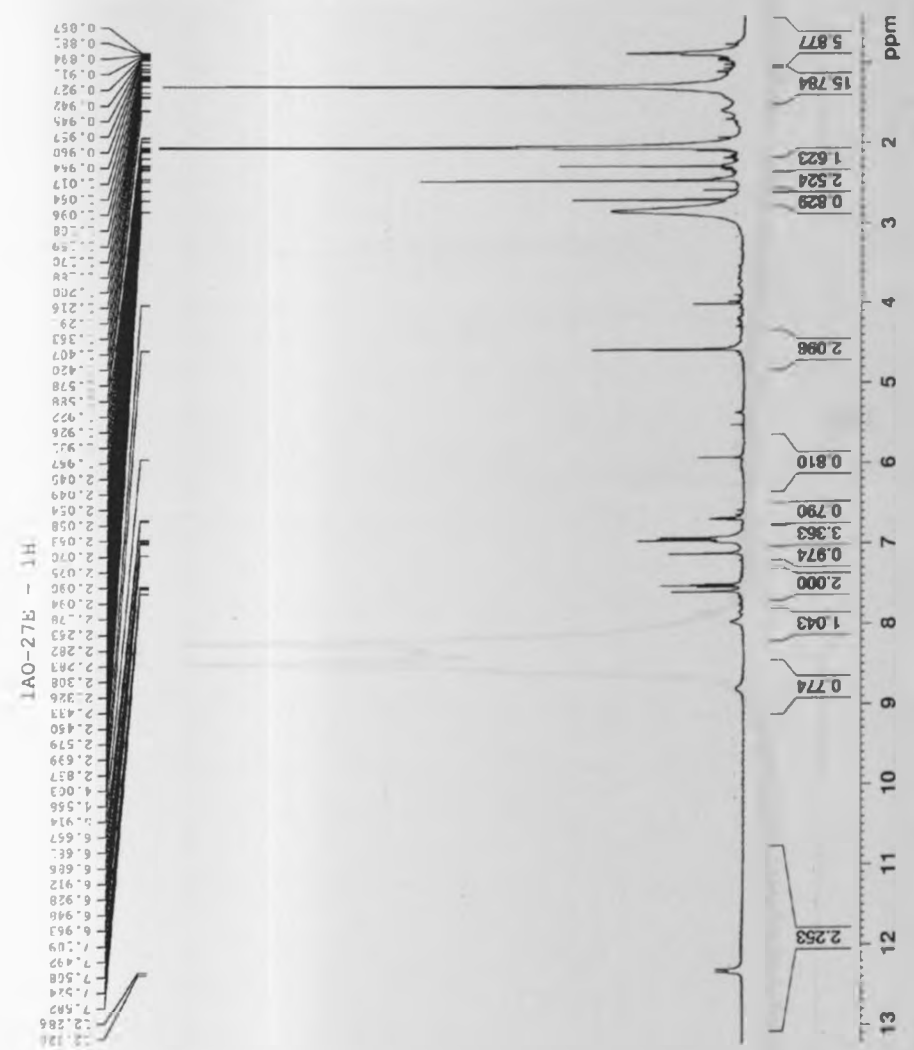


^{13}C NMR SPECTRUM FOR COMPOUND 11 (SOLVENT: ACETONE-d_6, 125 MHz)

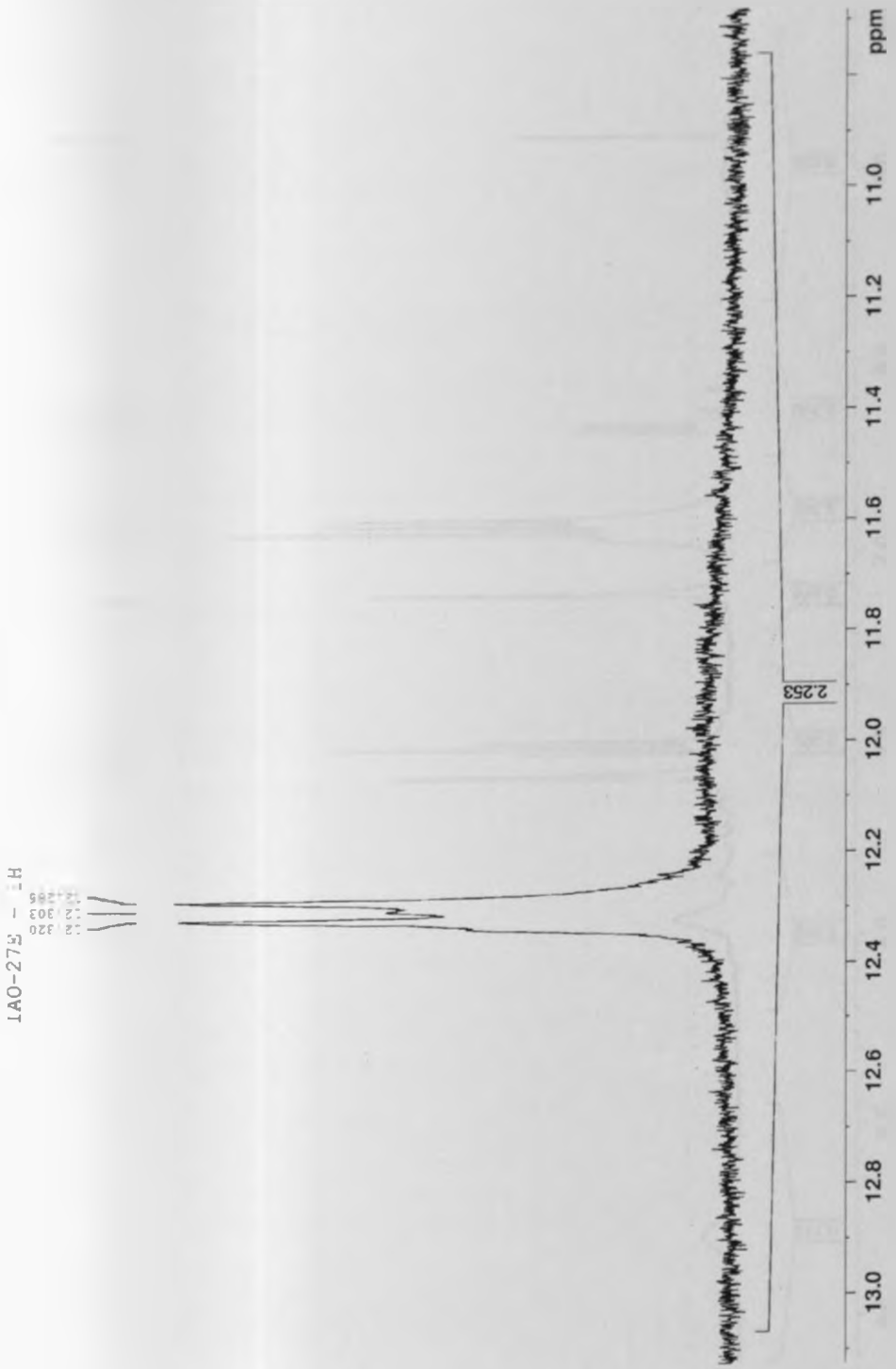


1AO-27E - 13C

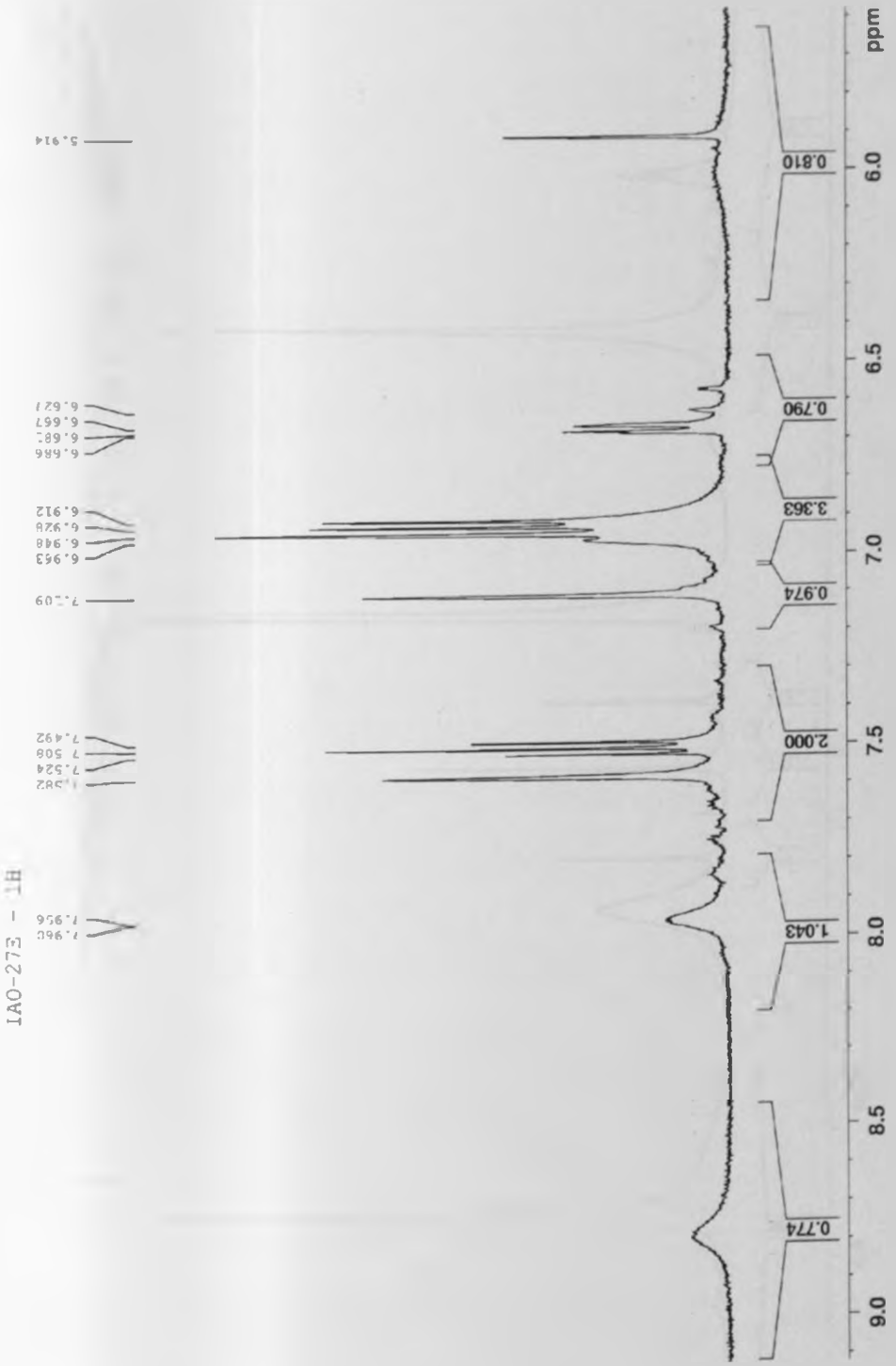
^{1}H NMR SPECTRUM FOR COMPOUND 11 (SOLVENT: $CDCl_3$, 500 MHz)



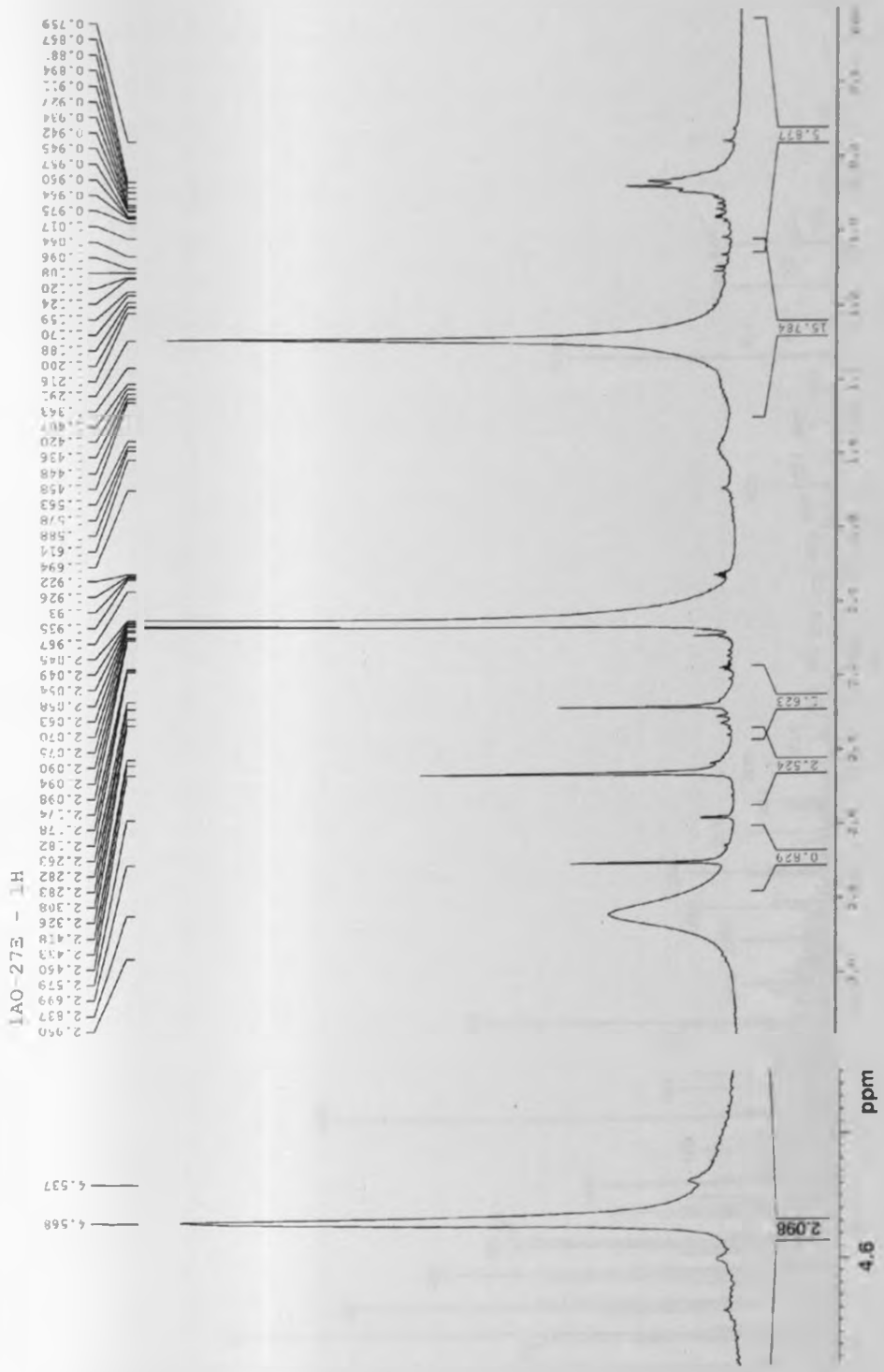
^{1}H NMR SPECTRUM FOR COMPOUND 11 (SOLVENT: $CDCl_3$, 500 MHz)



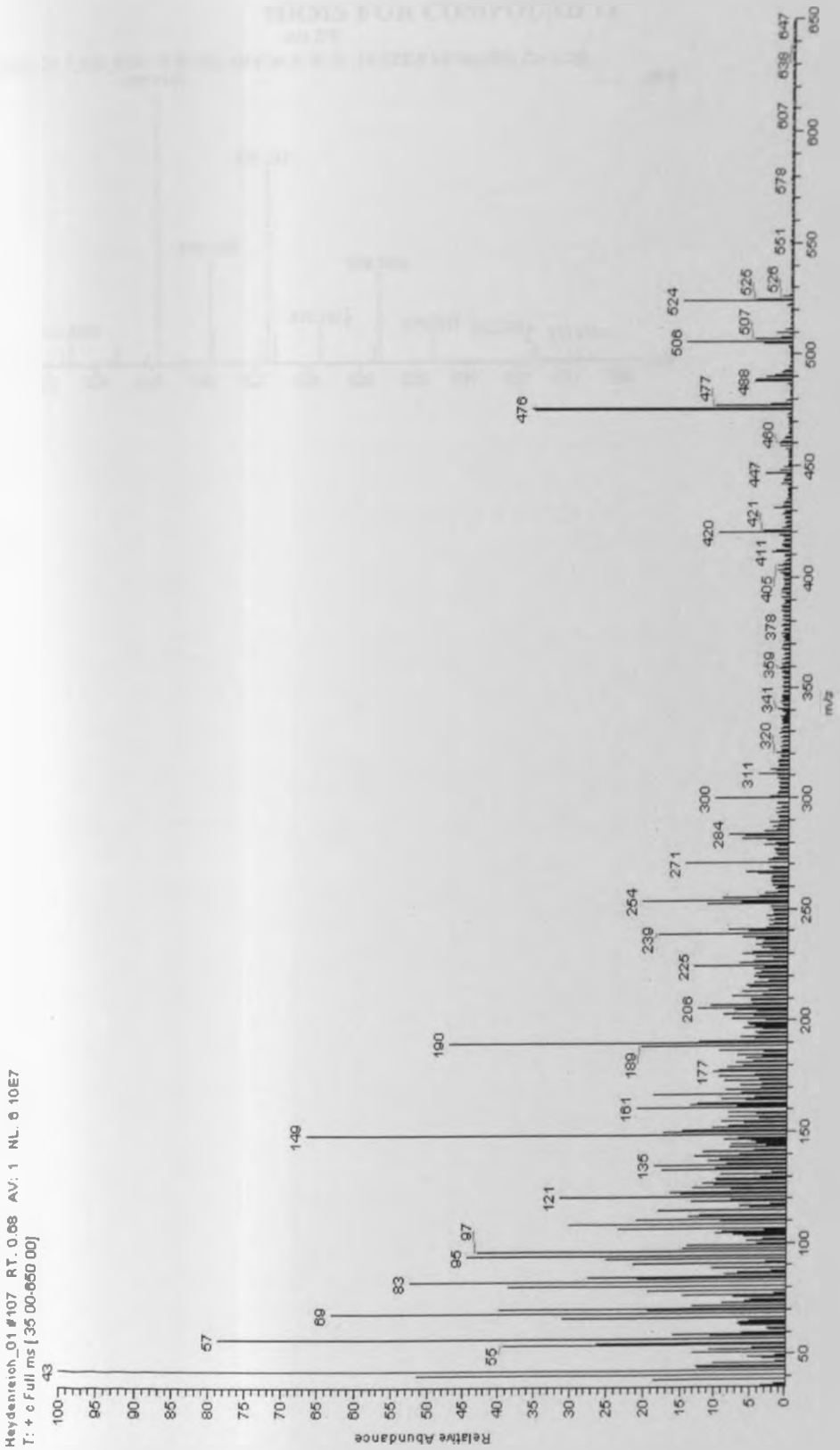
^{1}H NMR SPECTRUM FOR COMPOUND 11 (SOLVENT: $CDCl_3$, 500 MHz)



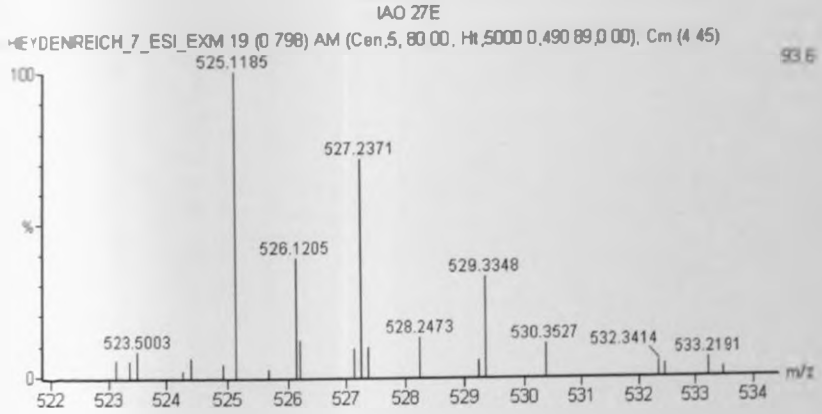
^{1}H NMR SPECTRUM FOR COMPOUND 11 (SOLVENT: $CDCl_3$, 500 MHz)



MS FOR COMPOUND 11

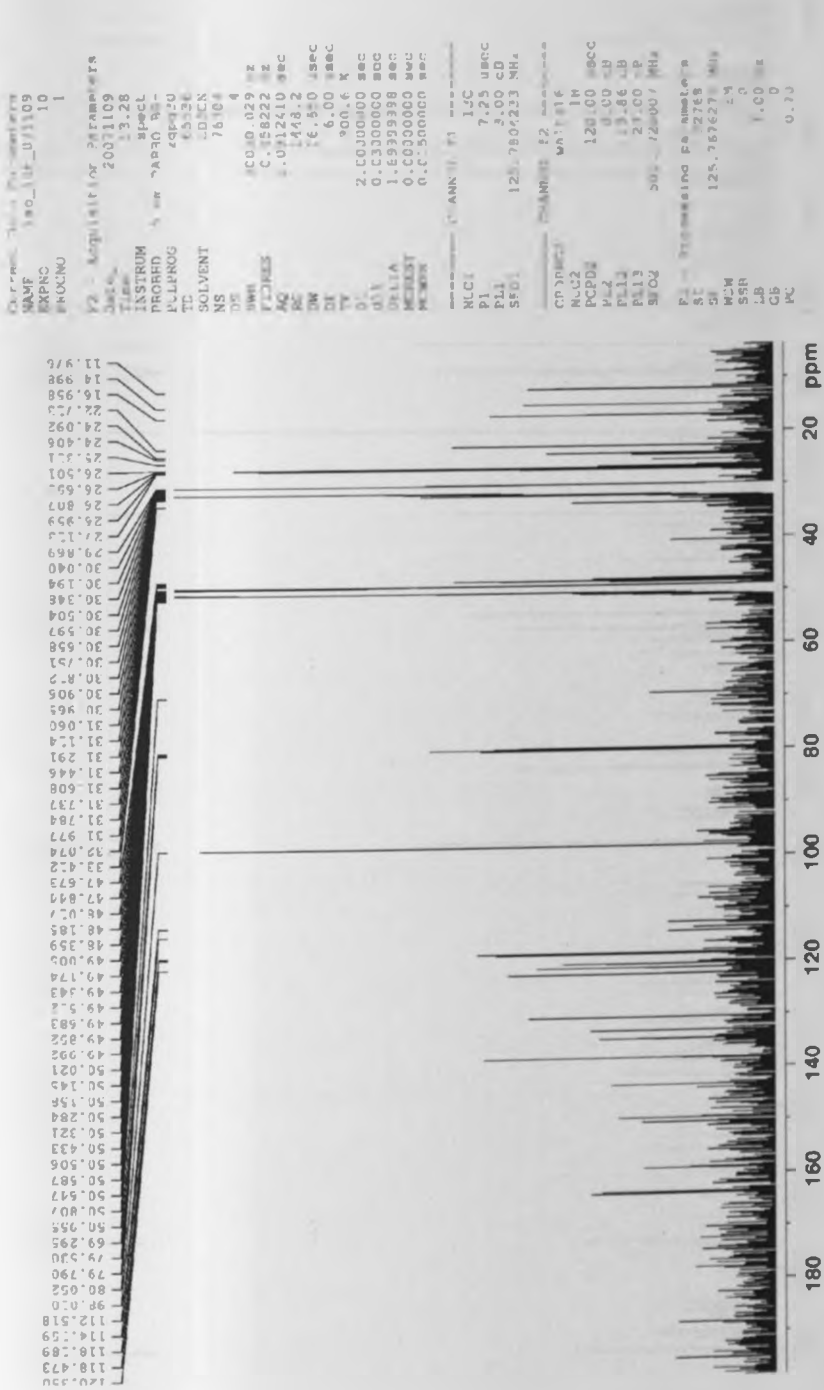


HRMS FOR COMPOUND 11

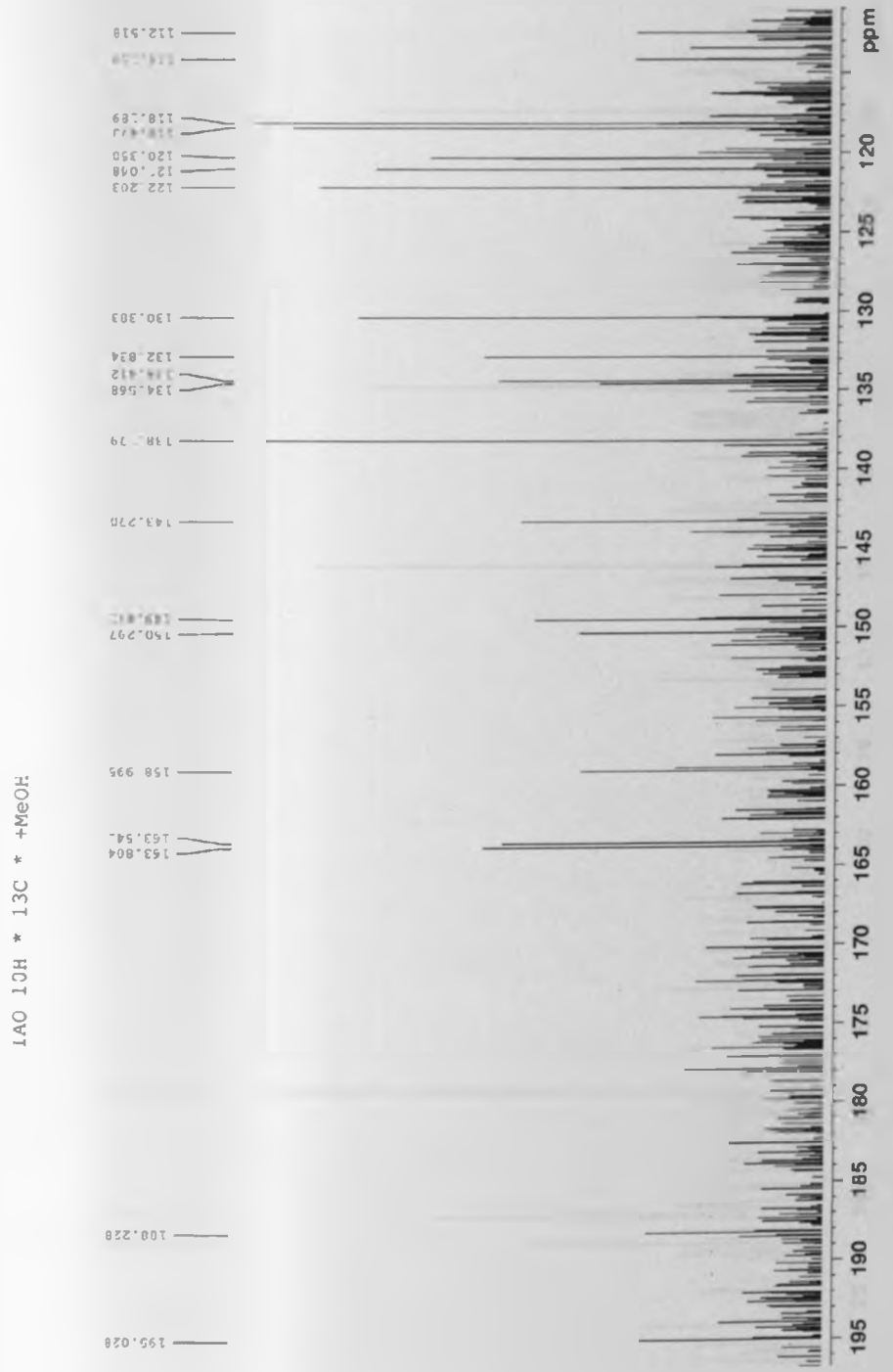


SPECTRA FOR COMPOUND 12

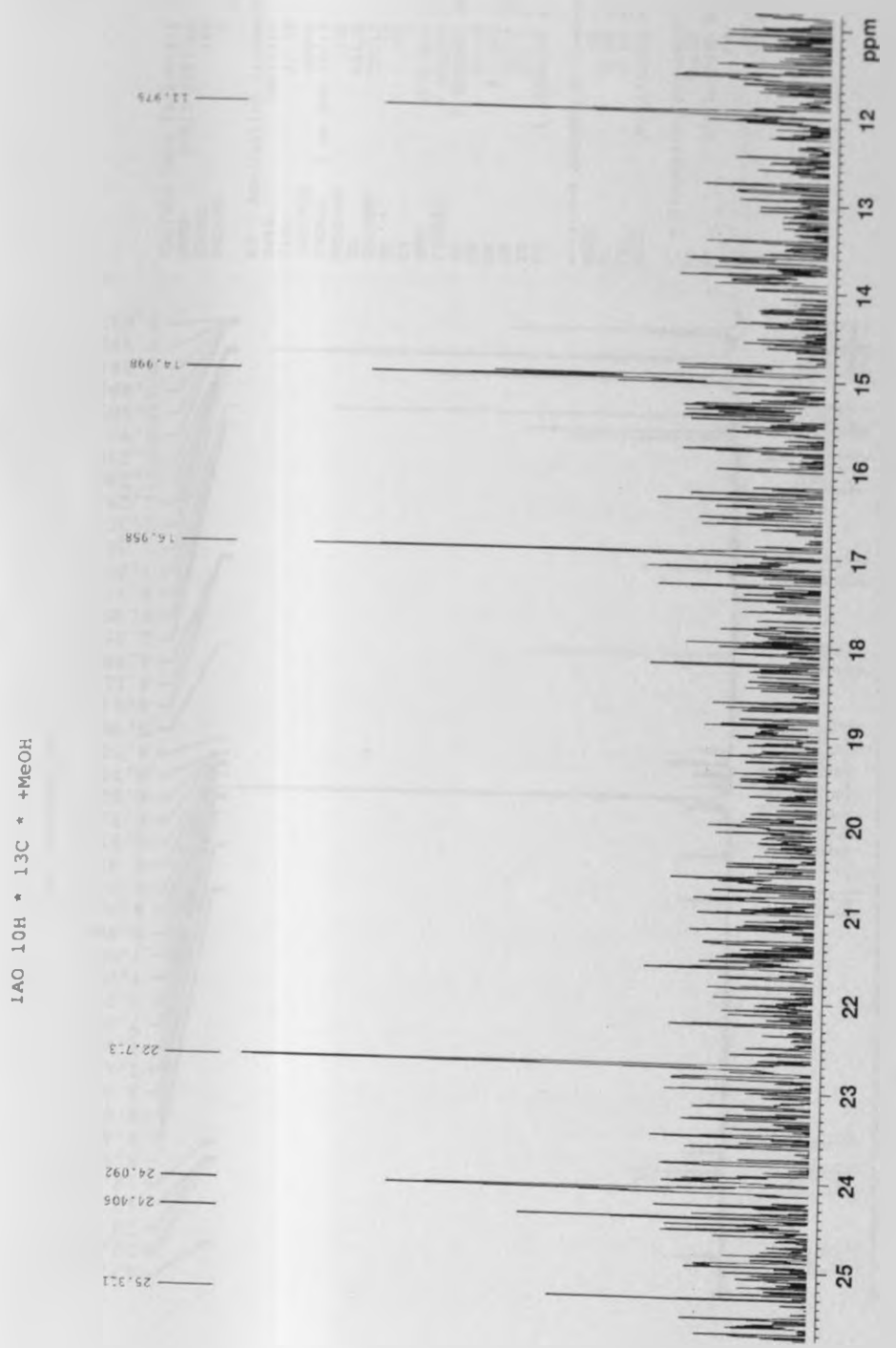
^{13}C NMR SPECTRUM FOR COMPOUND 12 (SOLVENT: ACETONE-d_6+MeOH-d_4, 125MHz)



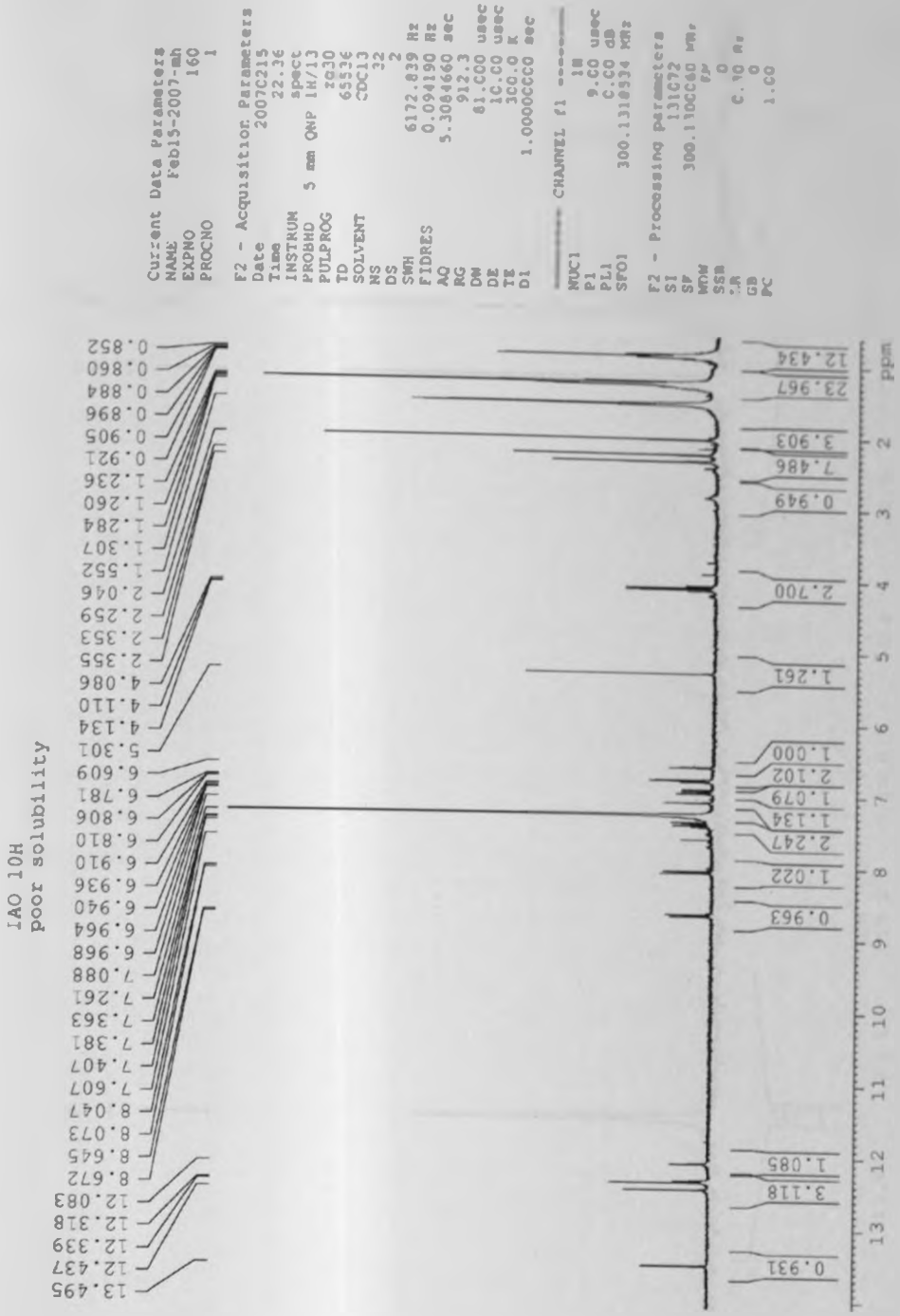
^{13}C NMR SPECTRUM FOR COMPOUND 12 (SOLVENT: ACETONE-d_6+MeOH-d_4, 125MHz)



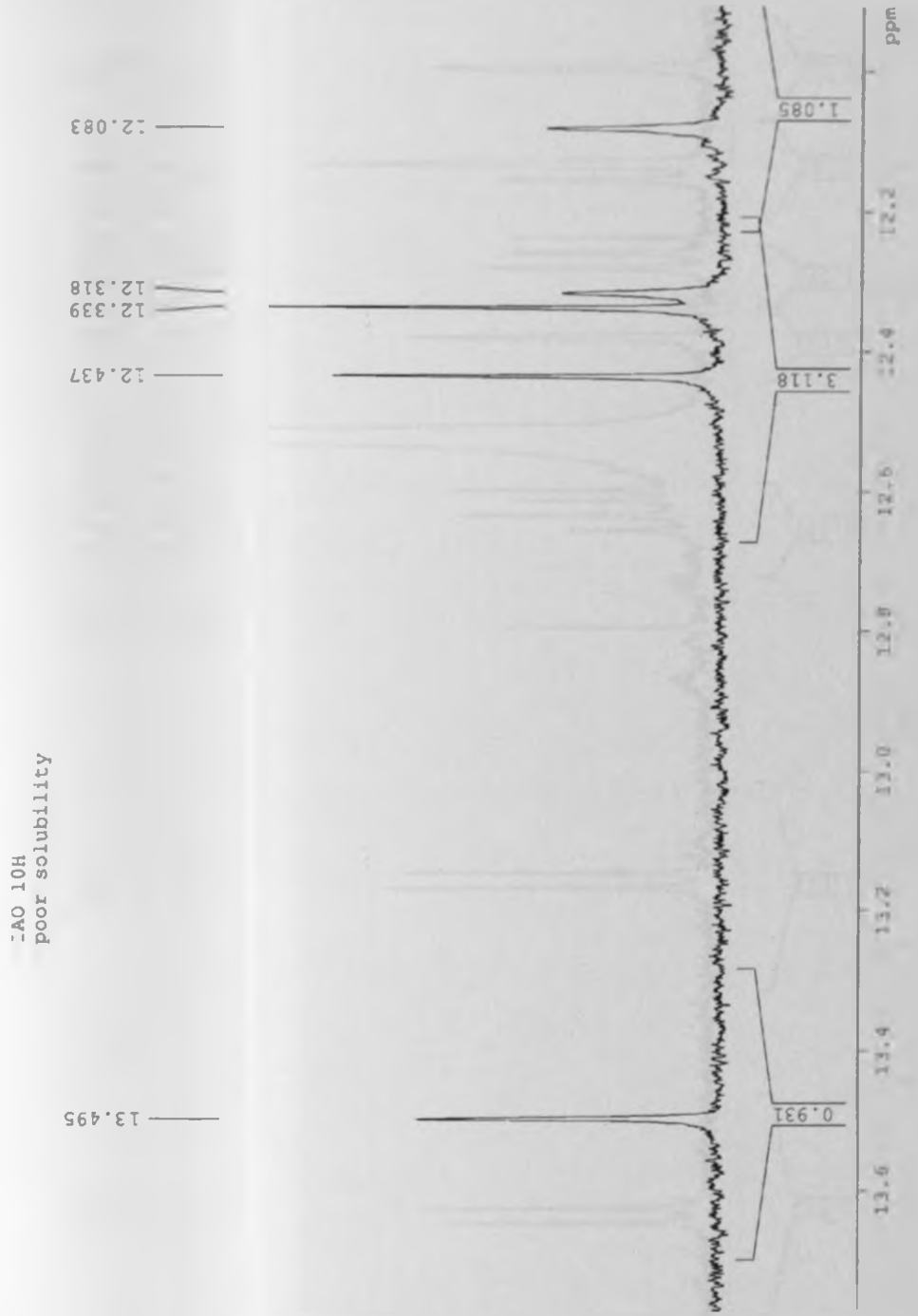
^{13}C NMR SPECTRUM FOR COMPOUND 12 (SOLVENT: ACETONE-d_6+MeOH-d_4, 125MHz)



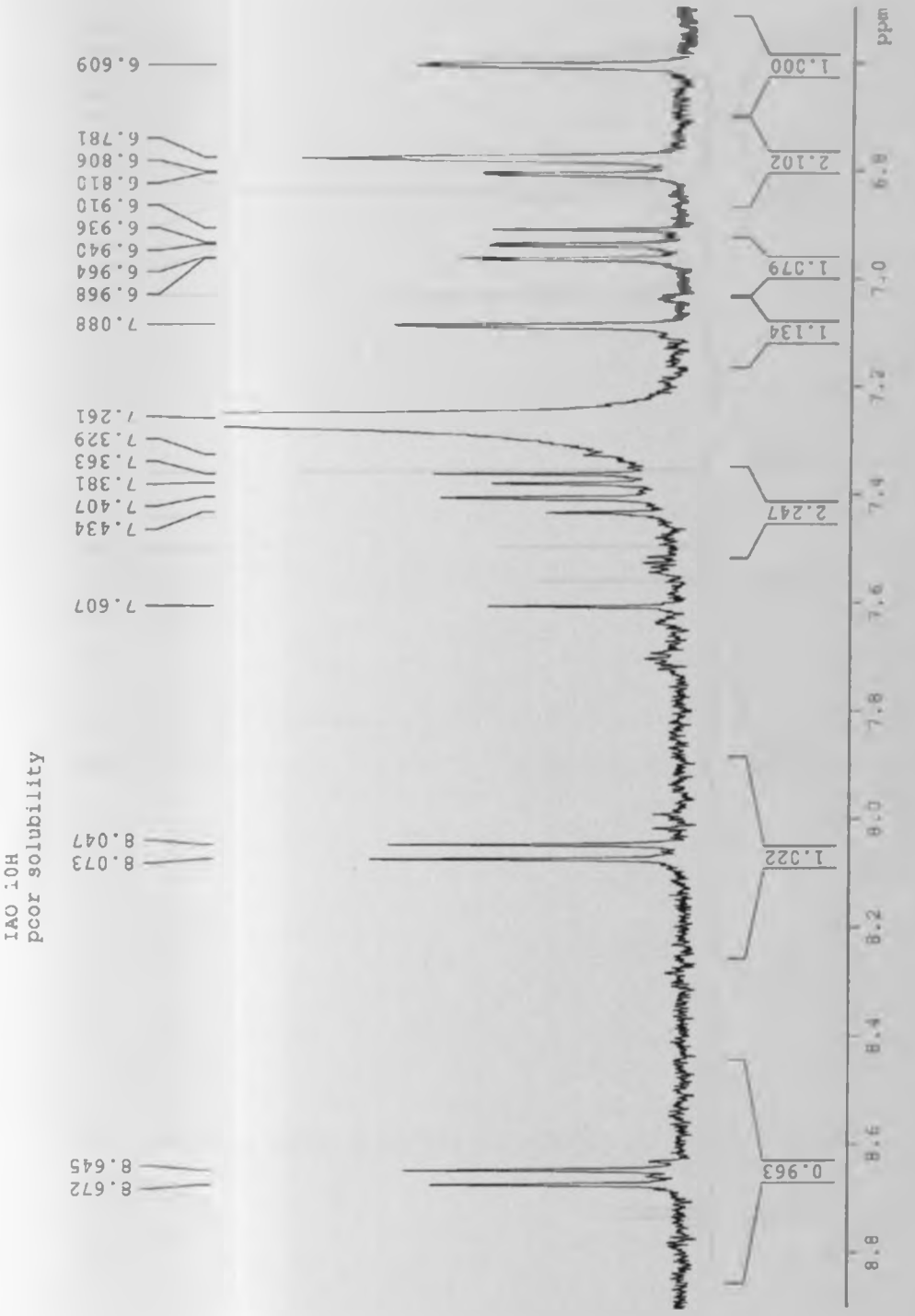
^{1}H NMR SPECTRUM FOR COMPOUND 12 (SOLVENT: $CDCl_3$, 300 MHz)



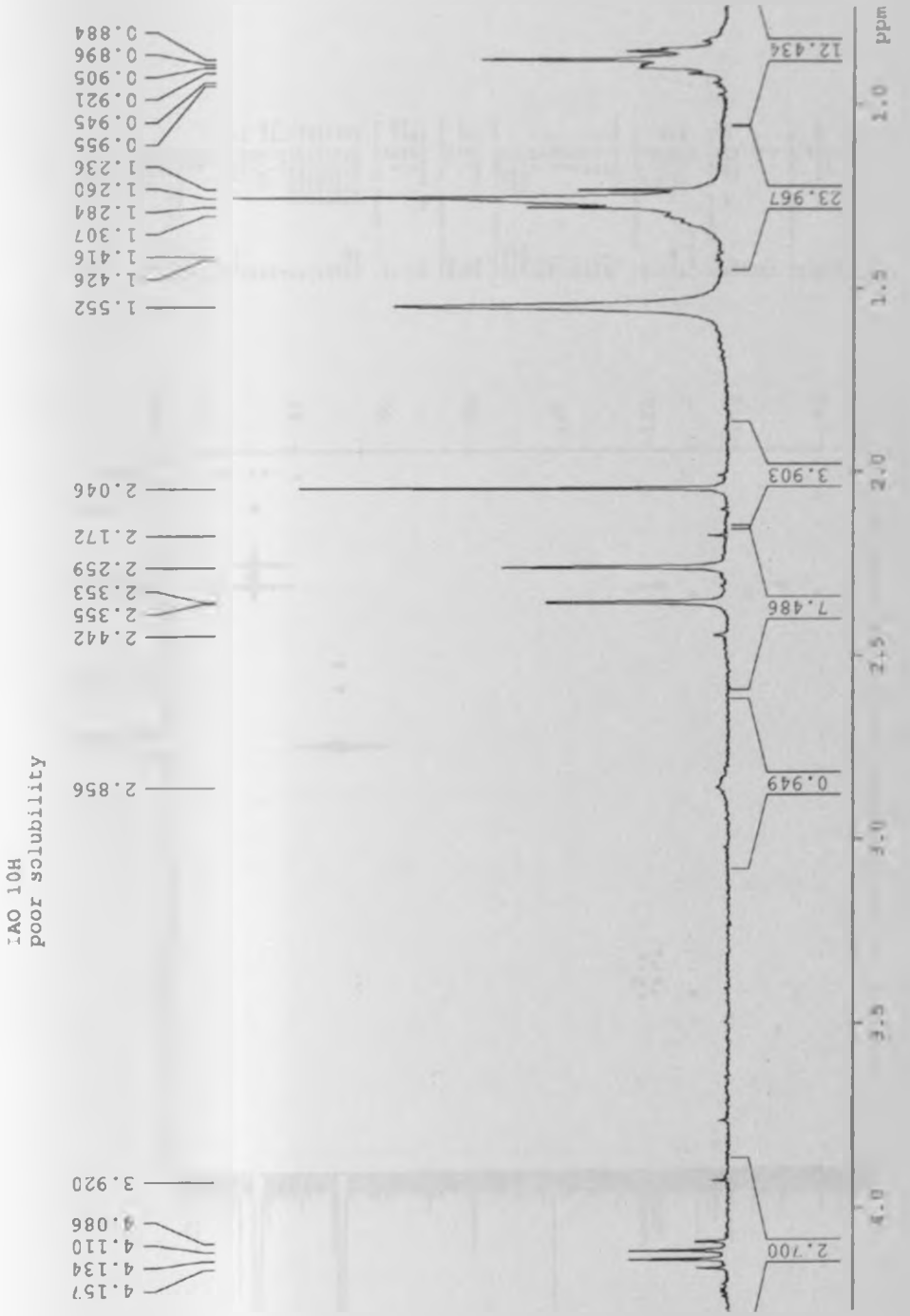
^{1}H NMR SPECTRUM FOR COMPOUND 12 (SOLVENT: $CDCl_3$, 300 MHz)



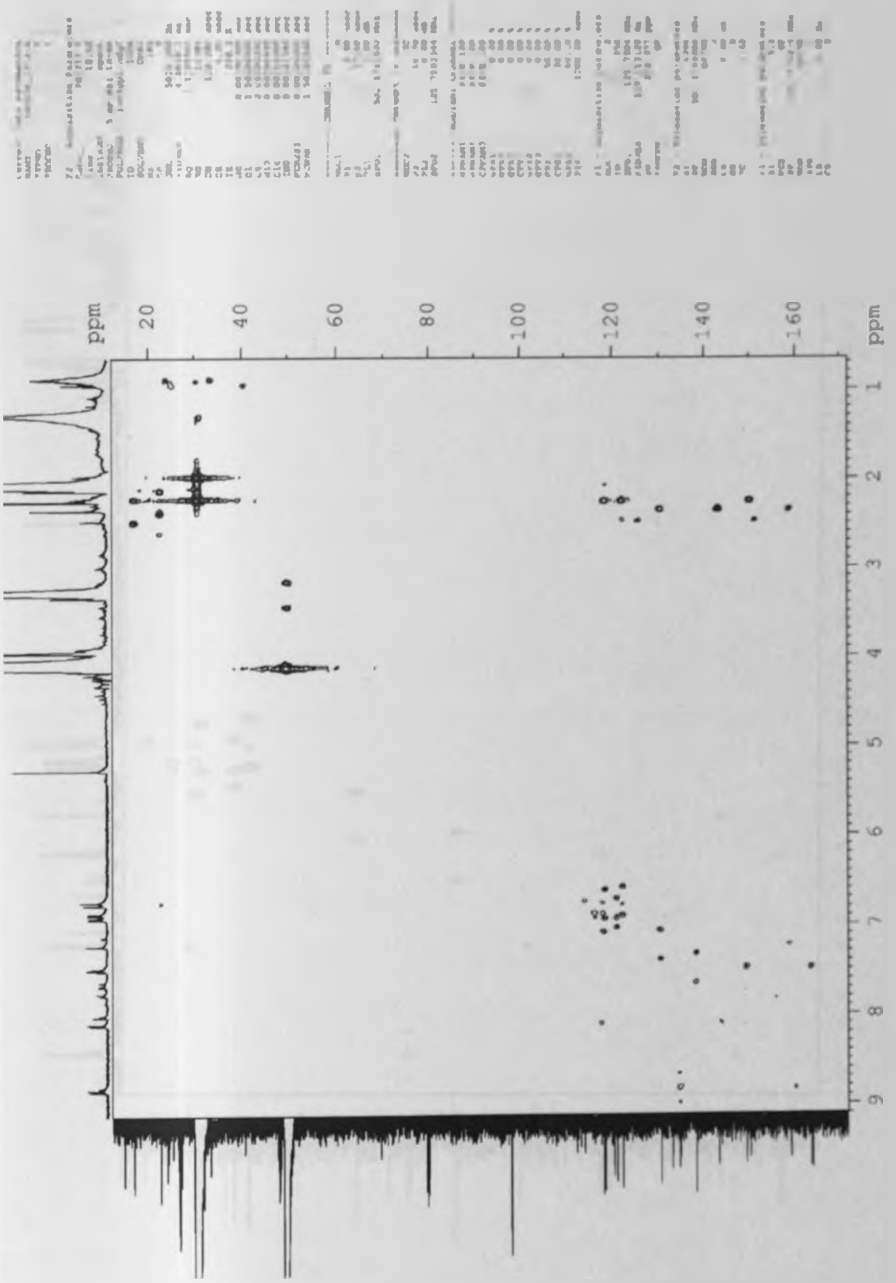
^{1}H NMR SPECTRUM FOR COMPOUND 12 (SOLVENT: $CDCl_3$, 300 MHz)



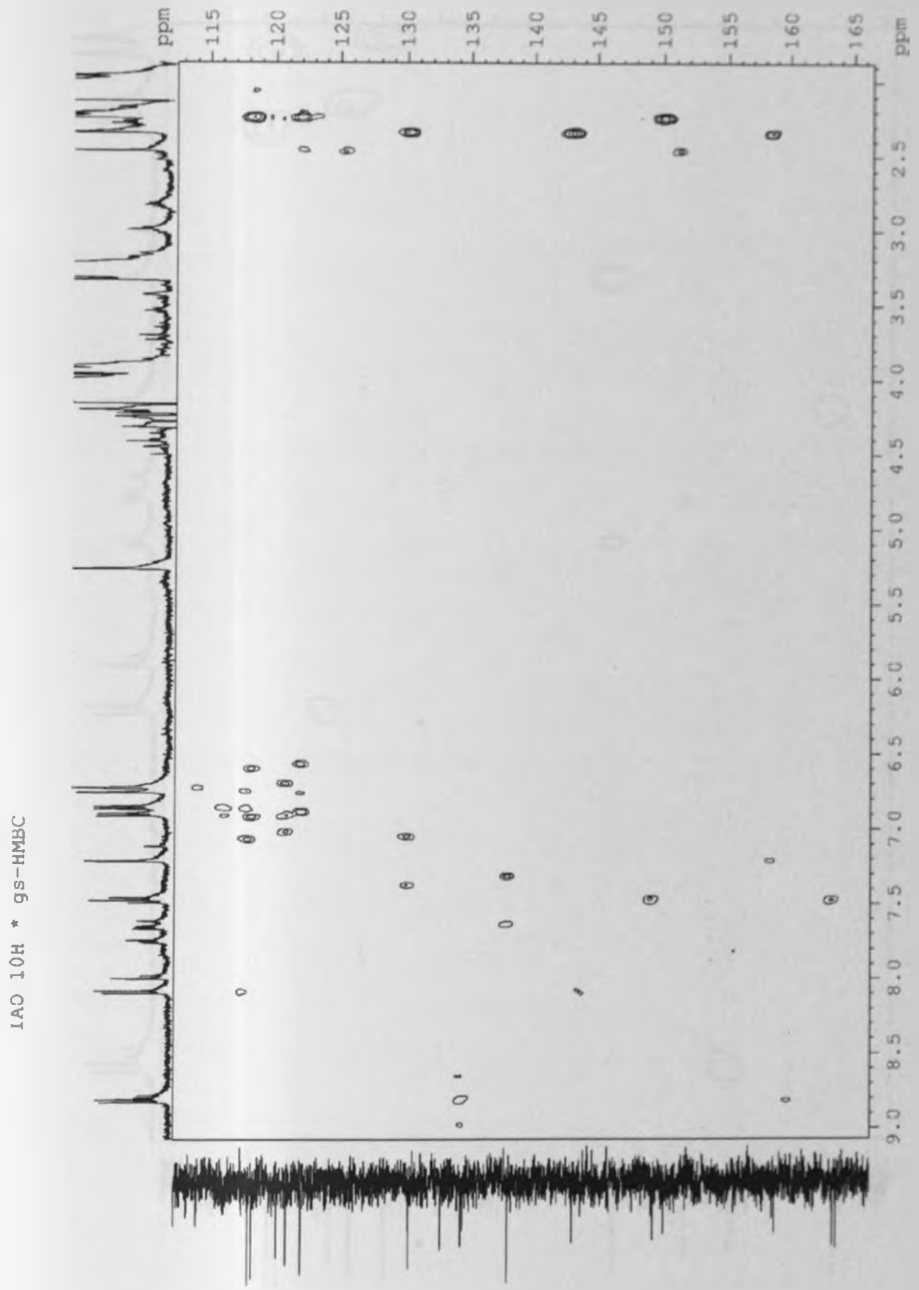
^{1}H NMR SPECTRUM FOR COMPOUND 12 (SOLVENT: $CDCl_3$, 300 MHz)



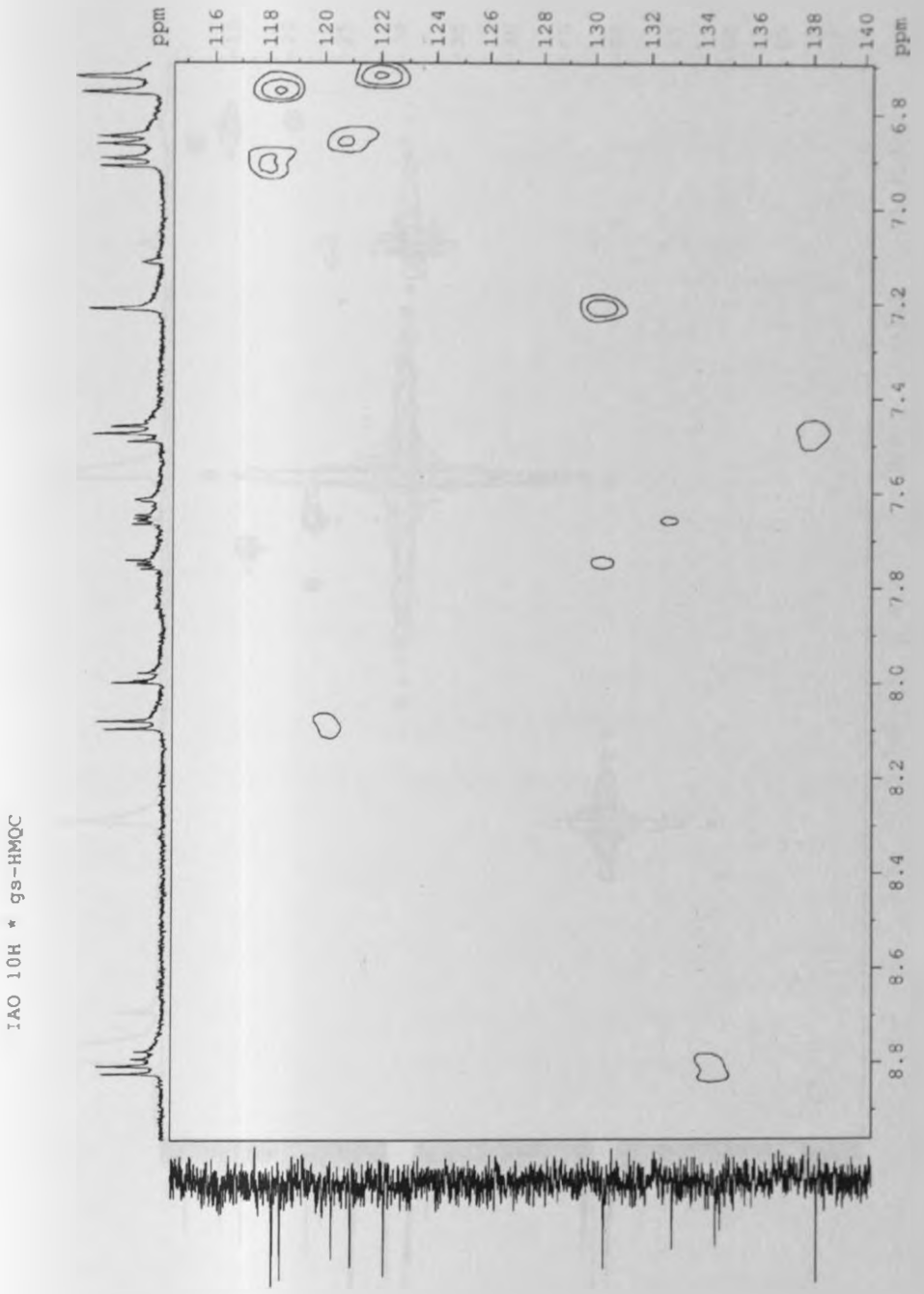
HMBC SPECTRUM FOR COMPOUND 12 (SOLVENT: ACETONE-d_6+MeOH-d_4, ^{1}H-500 and ^{13}C-125 MHz)



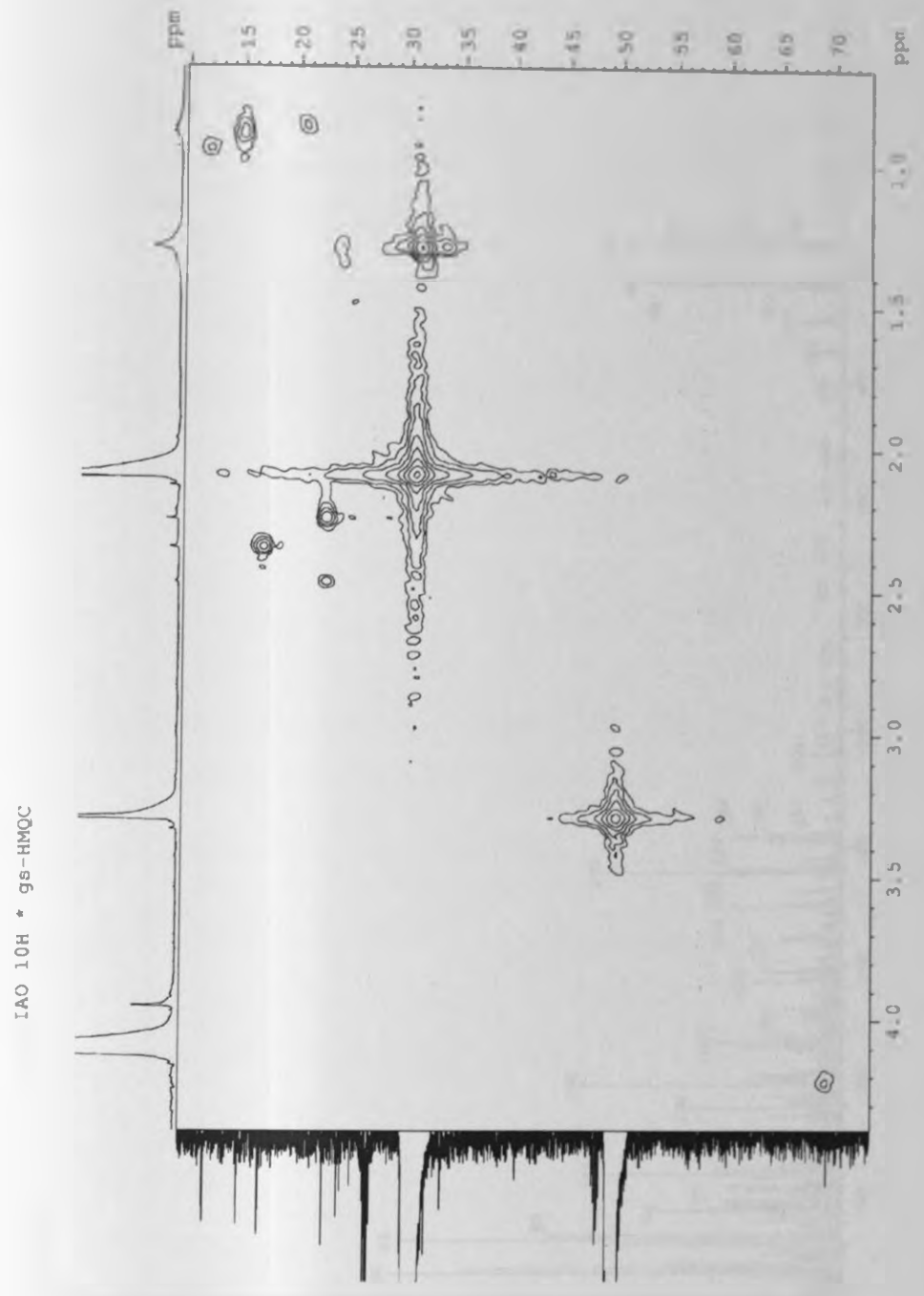
HMBC SPECTRUM FOR COMPOUND 12 (SOLVENT: ACETONE-d_6+MeOH-d_4, ^{1}H-500 and ^{13}C-125 MHz)



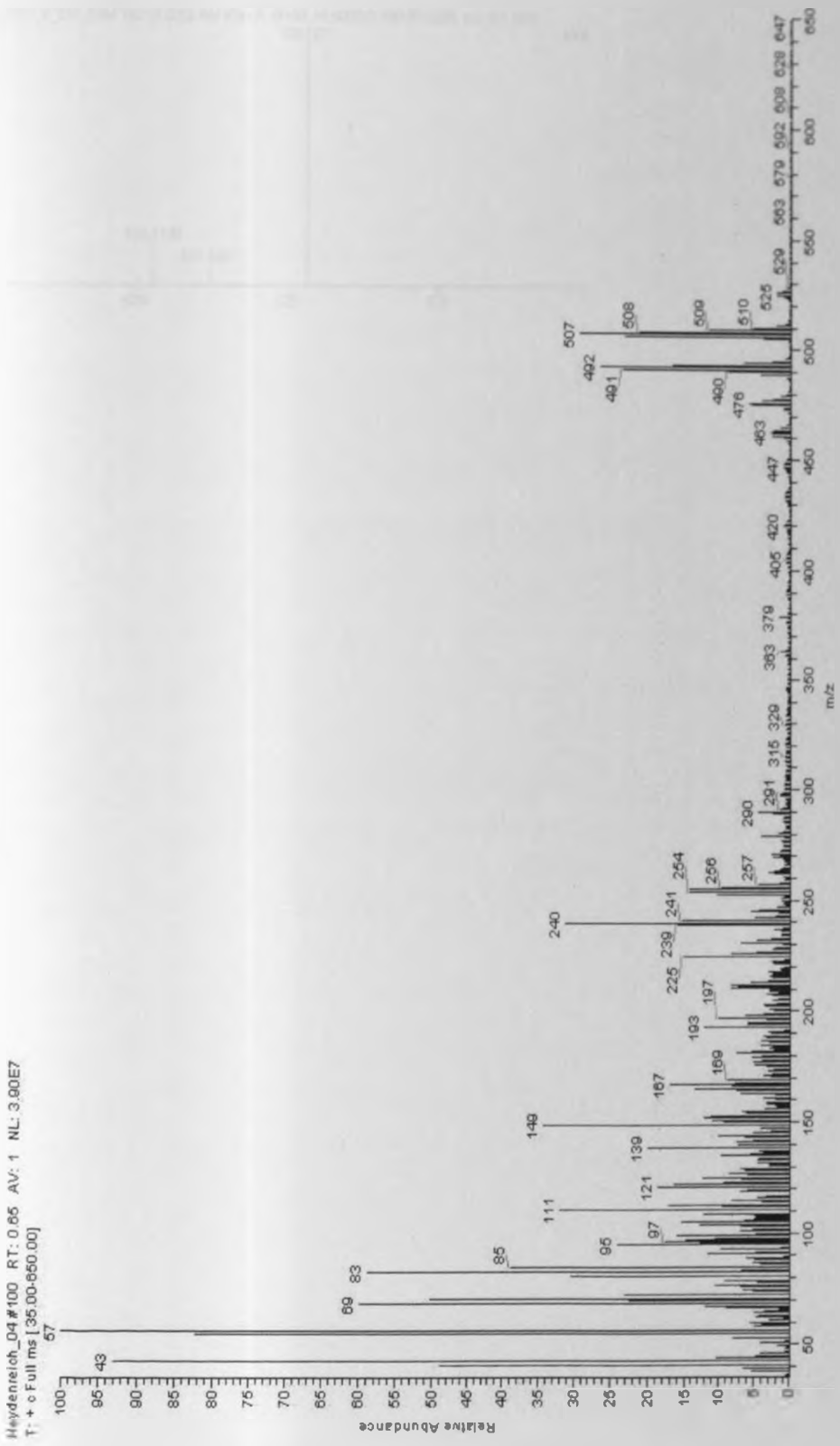
HMQC SPECTRUM FOR COMPOUND 12 (SOLVENT: ACETONE-d_6+MeOH-d_4, ^{1}H-500 and ^{13}C-125 MHz)



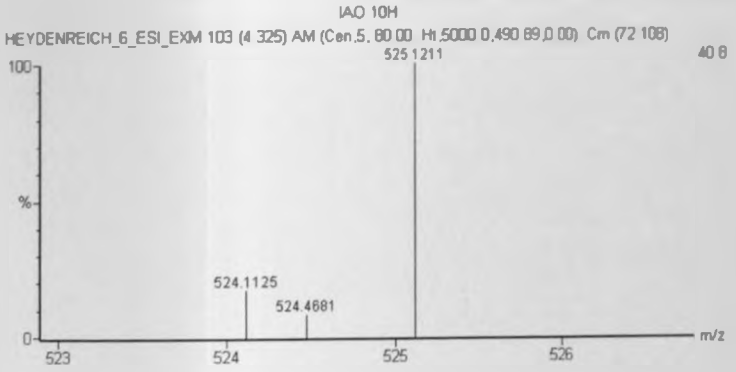
HMQC SPECTRUM FOR COMPOUND 12 (SOLVENT: ACETONE-d_6+MeOH-d_4, ^{1}H-500 and ^{13}C-125 MHz)



MS FOR COMPOUND 12

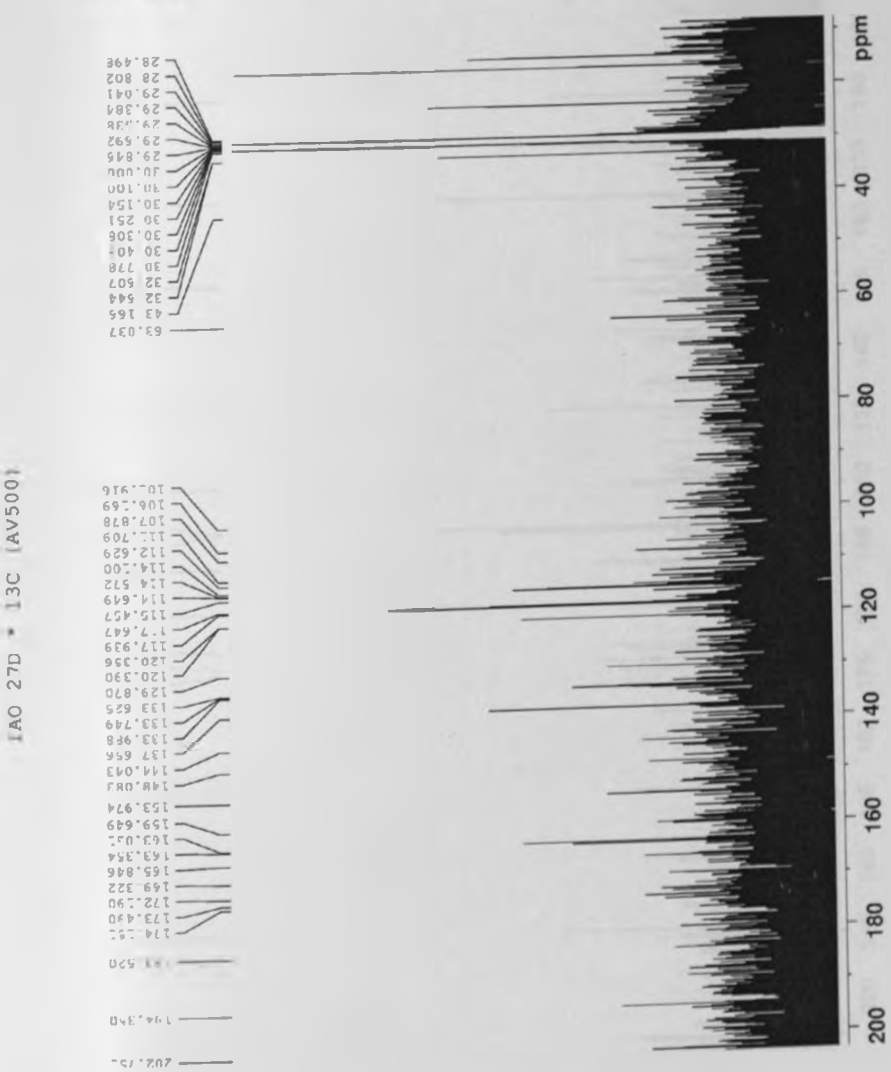


MS FOR COMPOUND 12

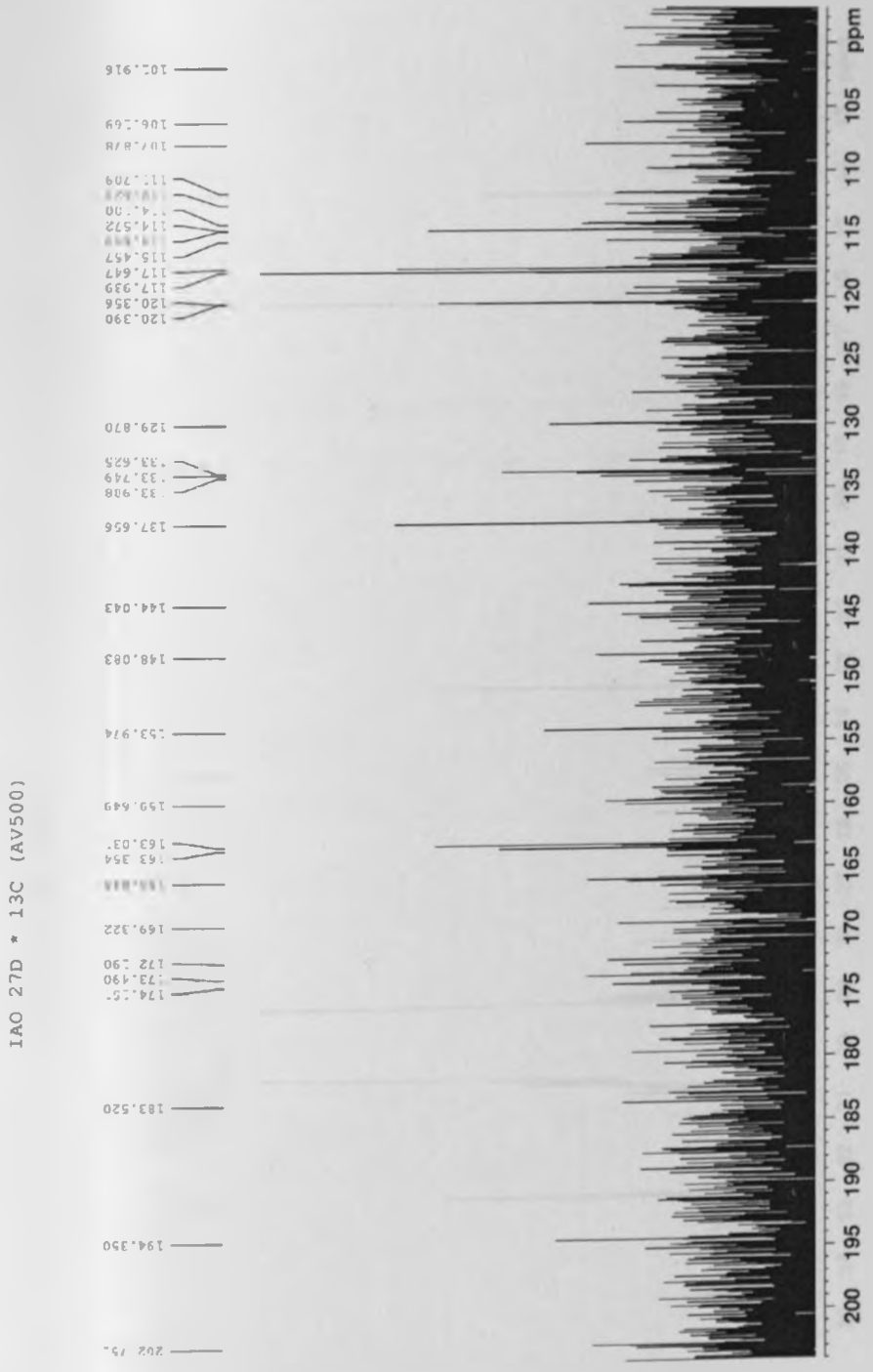


SPECTRA FOR COMPOUND 13

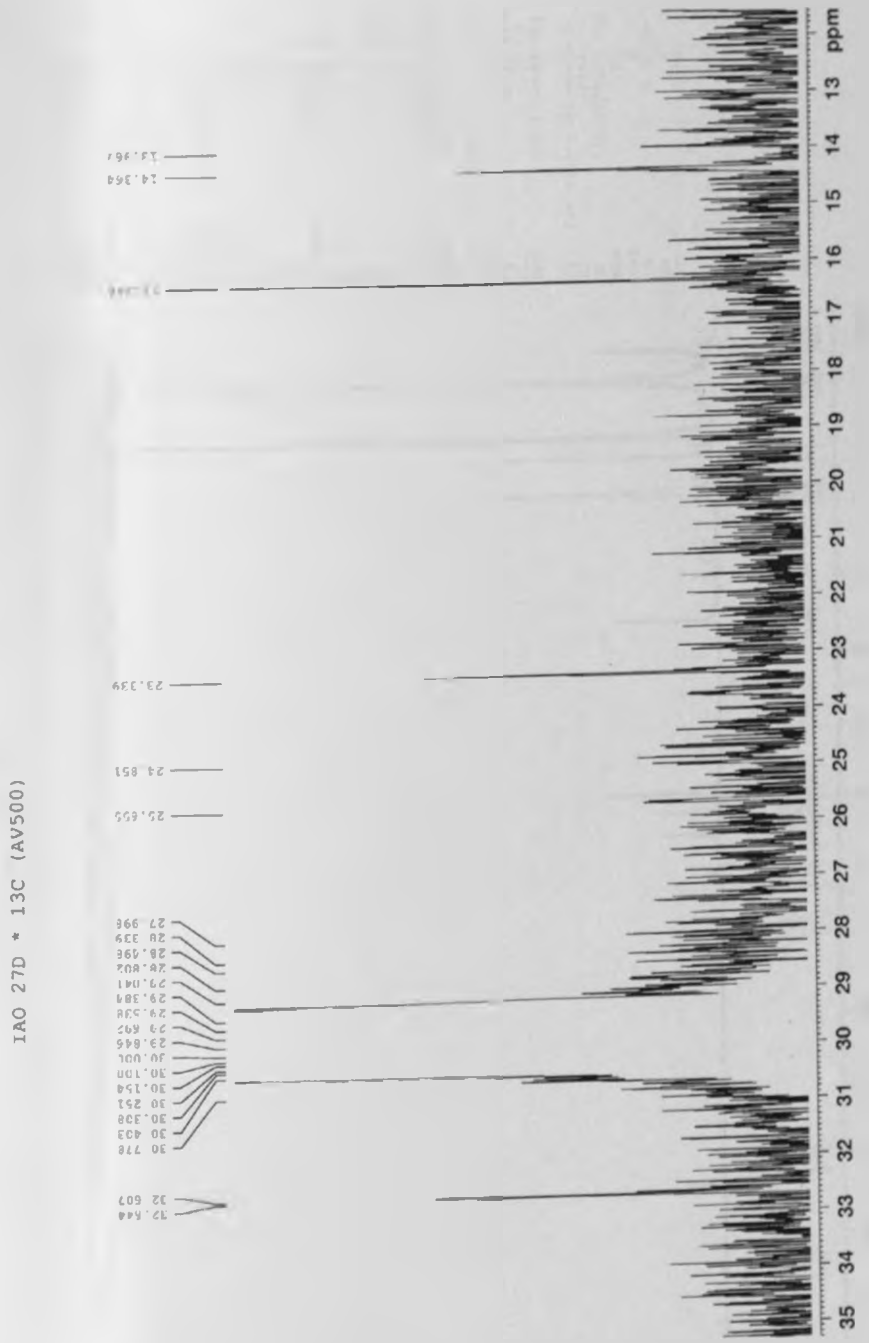
^{13}C NMR SPECTRUM FOR COMPOUND 13 (SOLVENT: ACETONE-d_6, 125 MHz)



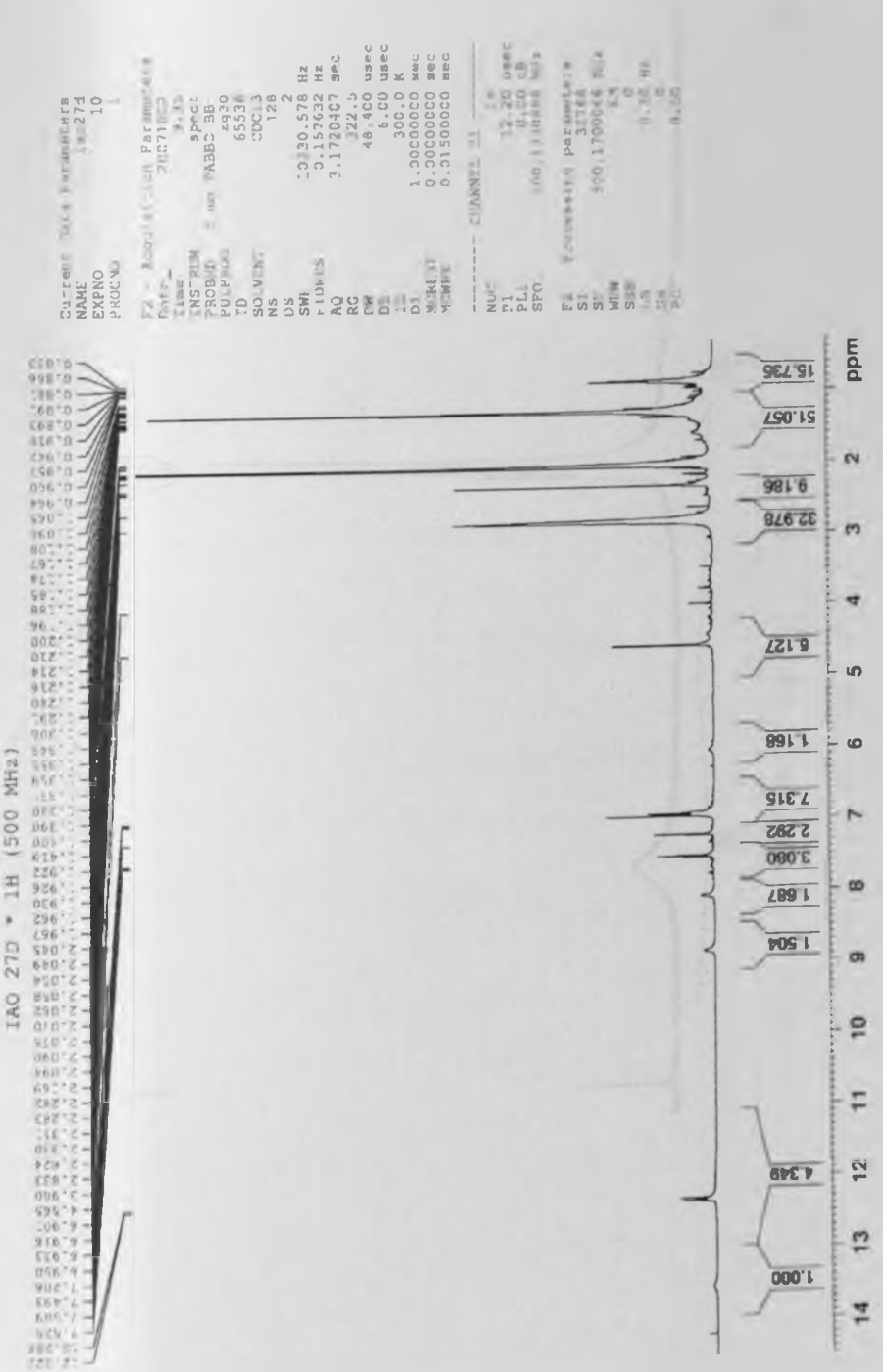
^{13}C NMR SPECTRUM FOR COMPOUND 13 (SOLVENT: ACETONE-d_6, 125 MHz)



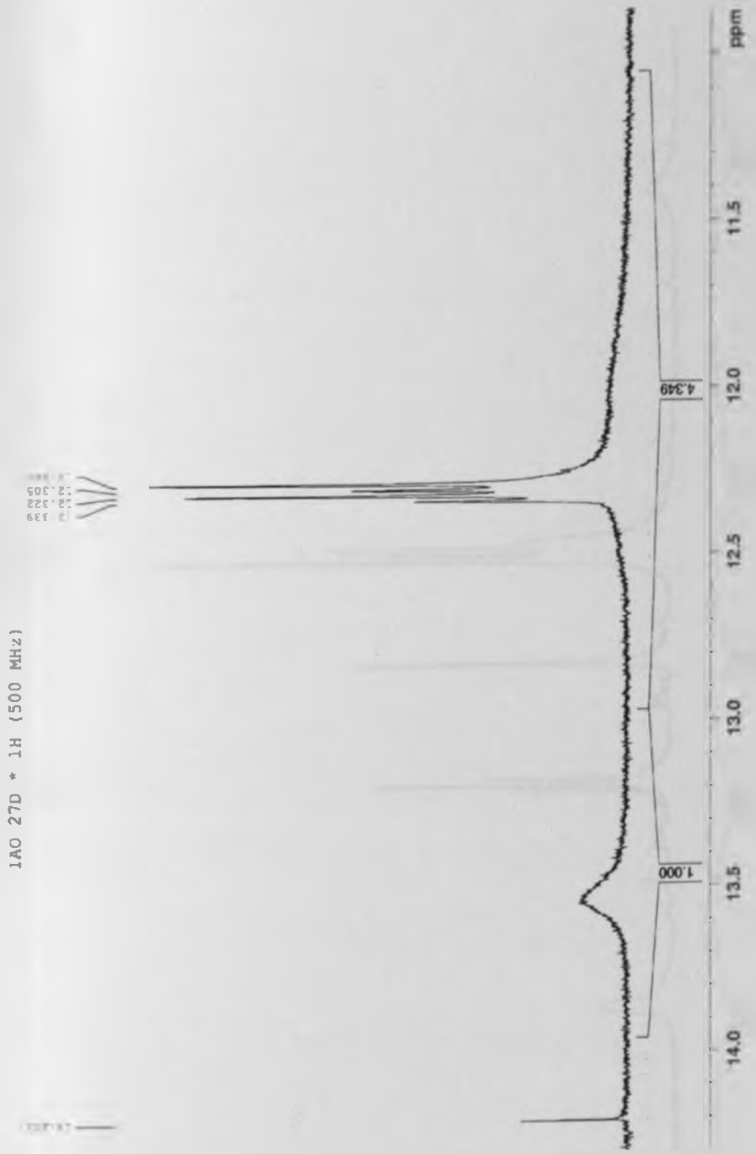
^{13}C NMR SPECTRUM FOR COMPOUND 13 (SOLVENT: ACETONE-d_6, 125 MHz)



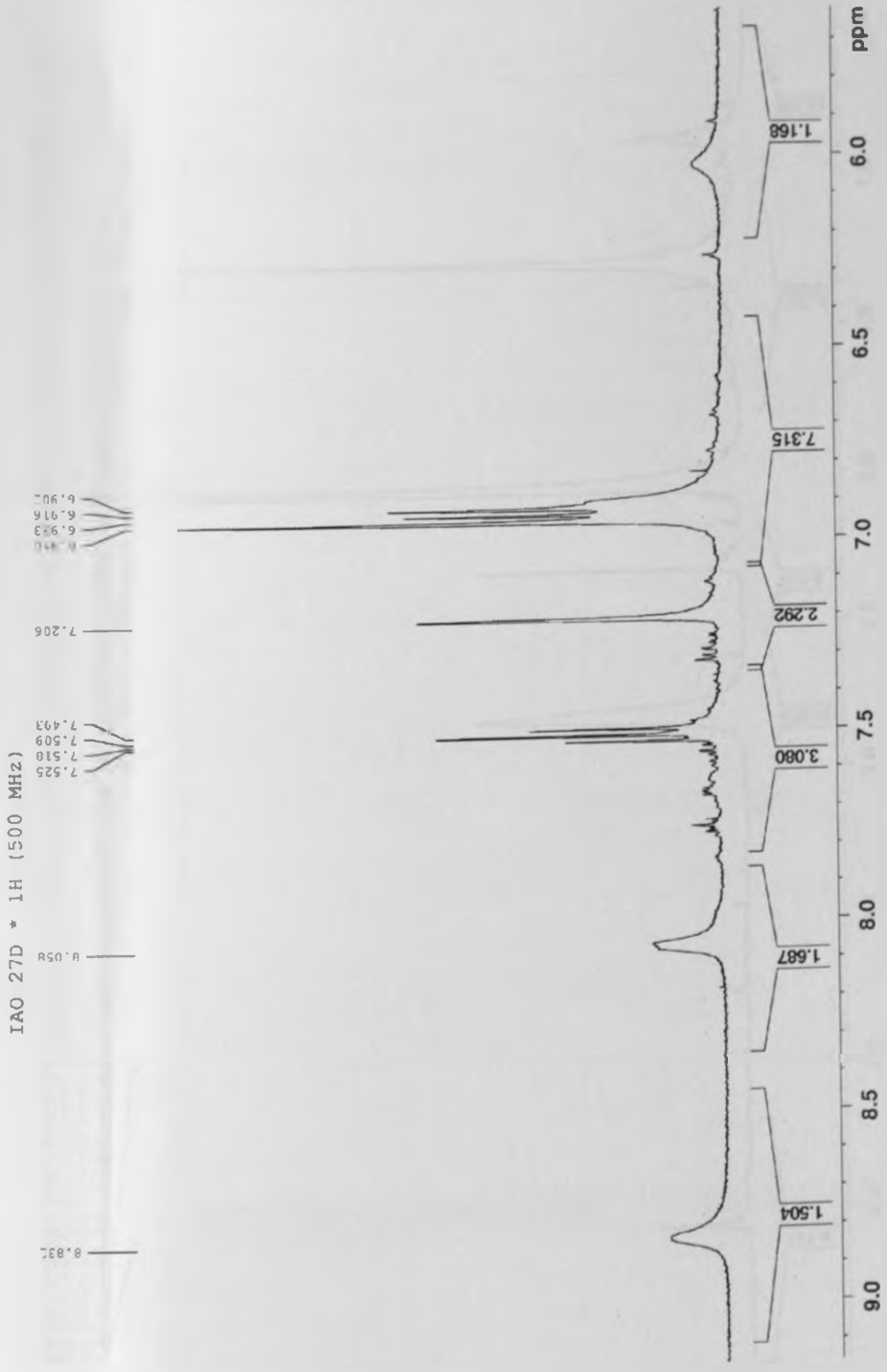
^{1}H NMR SPECTRUM FOR COMPOUND 13 (SOLVENT: $CDCl_3$, 500 MHz)



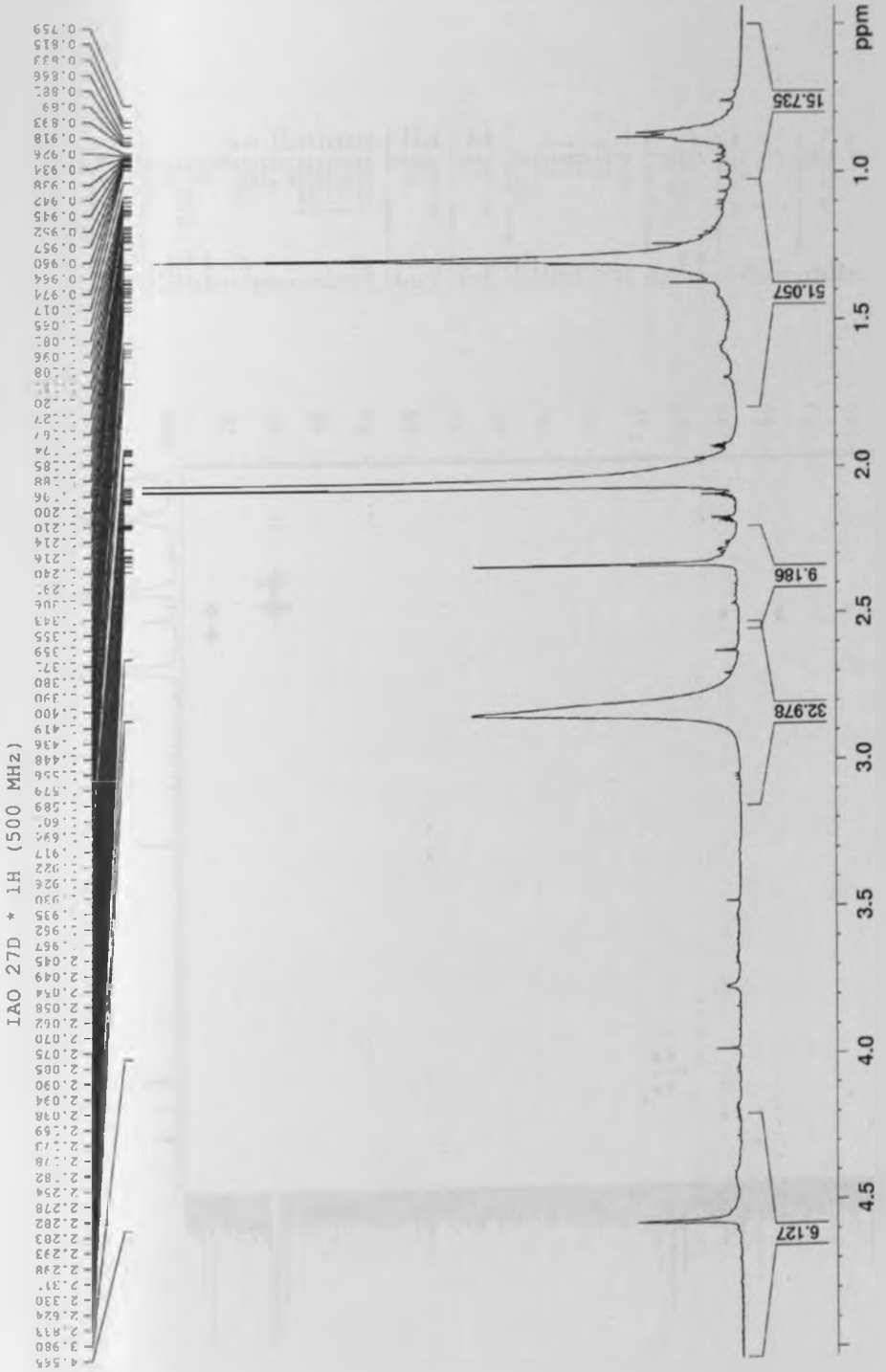
^{1}H NMR SPECTRUM FOR COMPOUND 13 (SOLVENT: $CDCl_3$, 500 MHz)



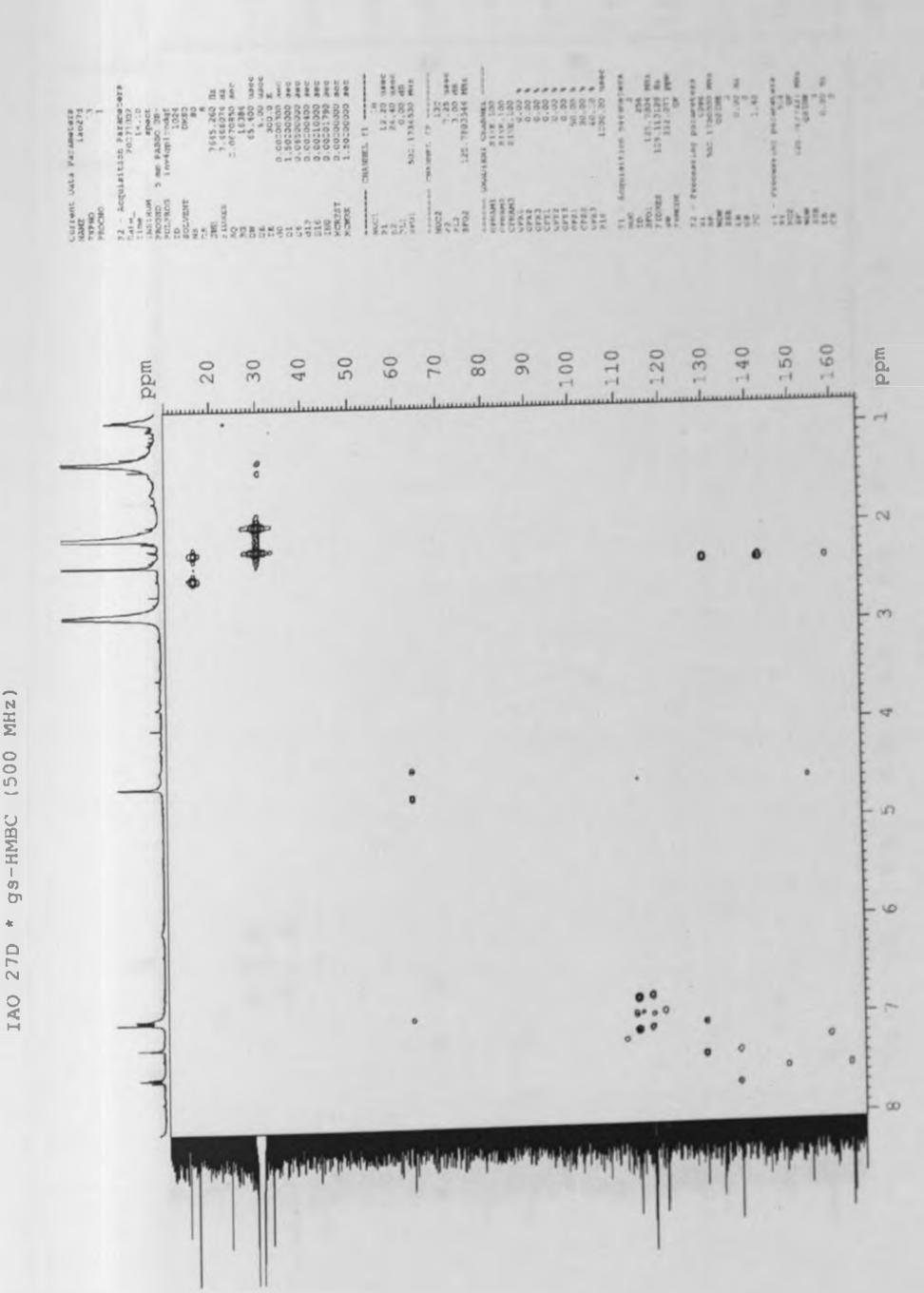
^{1}H NMR SPECTRUM FOR COMPOUND 13 (SOLVENT: $CDCl_3$, 500 MHz)



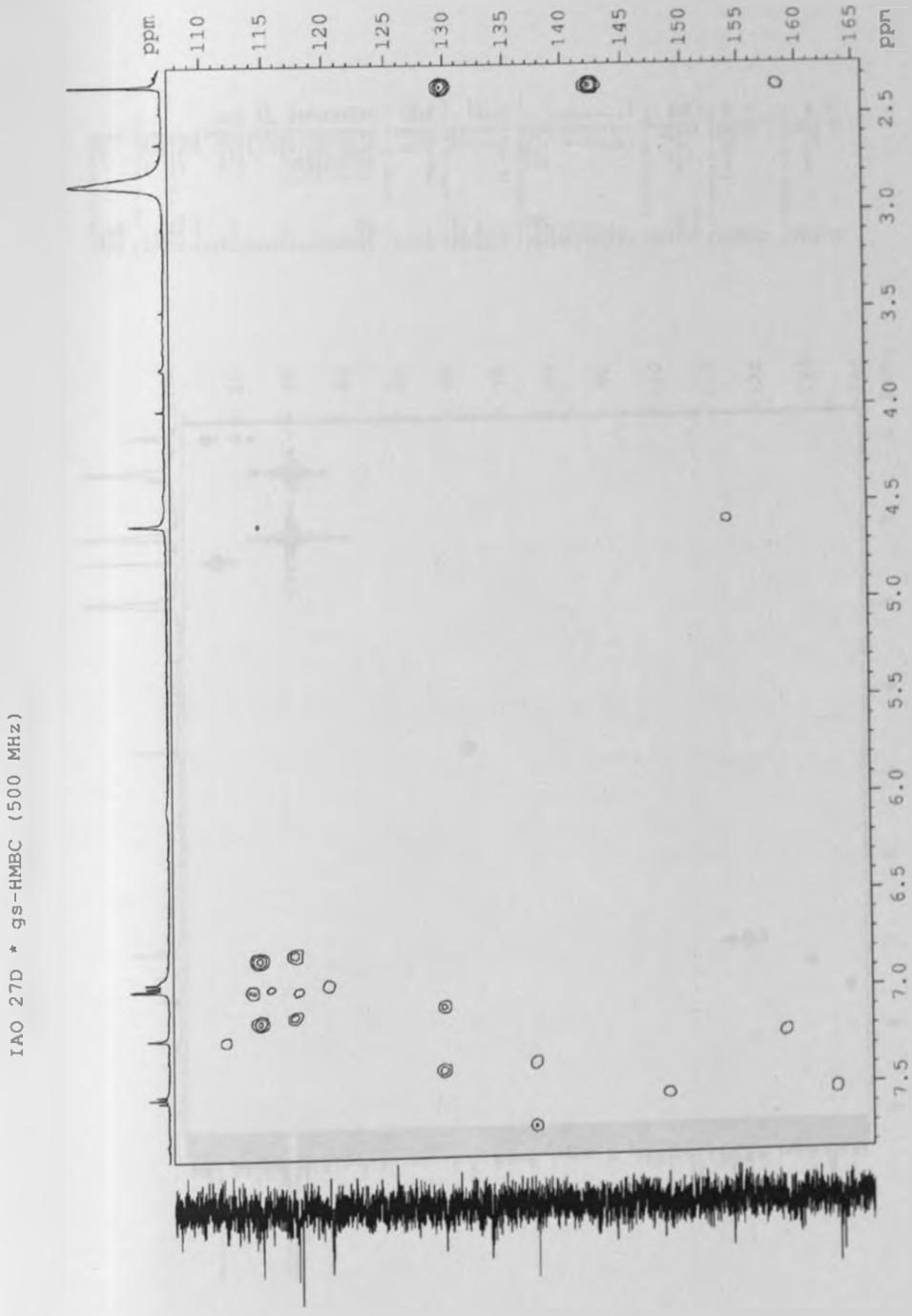
^{1}H NMR SPECTRUM FOR COMPOUND 13 (SOLVENT: $CDCl_3$, 500 MHz)



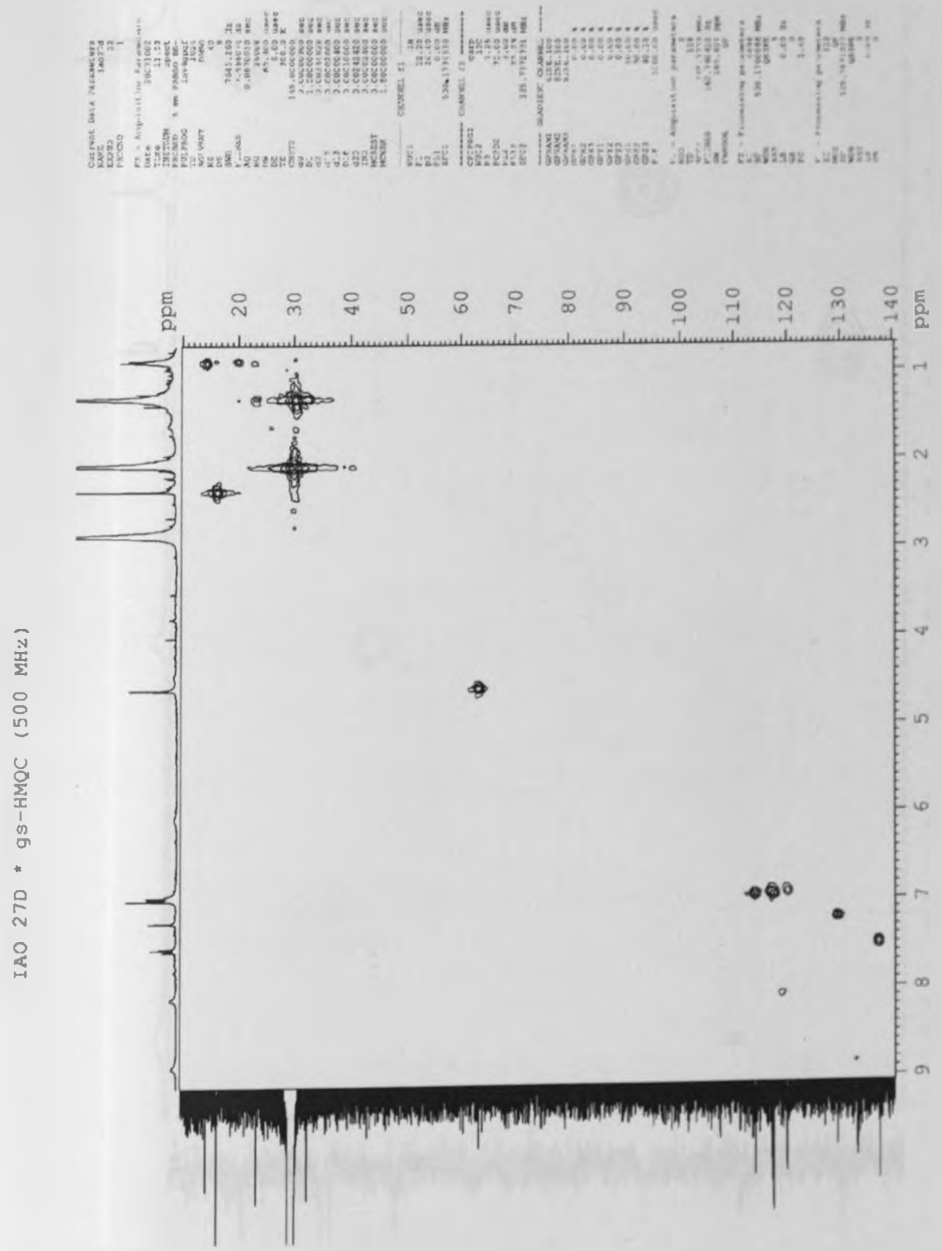
HMBC SPECTRUM FOR COMPOUND 13 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)



HMBC SPECTRUM FOR COMPOUND 13 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)



HMQC SPECTRUM FOR COMPOUND 13 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)



HMQC SPECTRUM FOR COMPOUND 13 (SOLVENT: ACETONE-d_6, ^{1}H-500 and ^{13}C-125 MHz)

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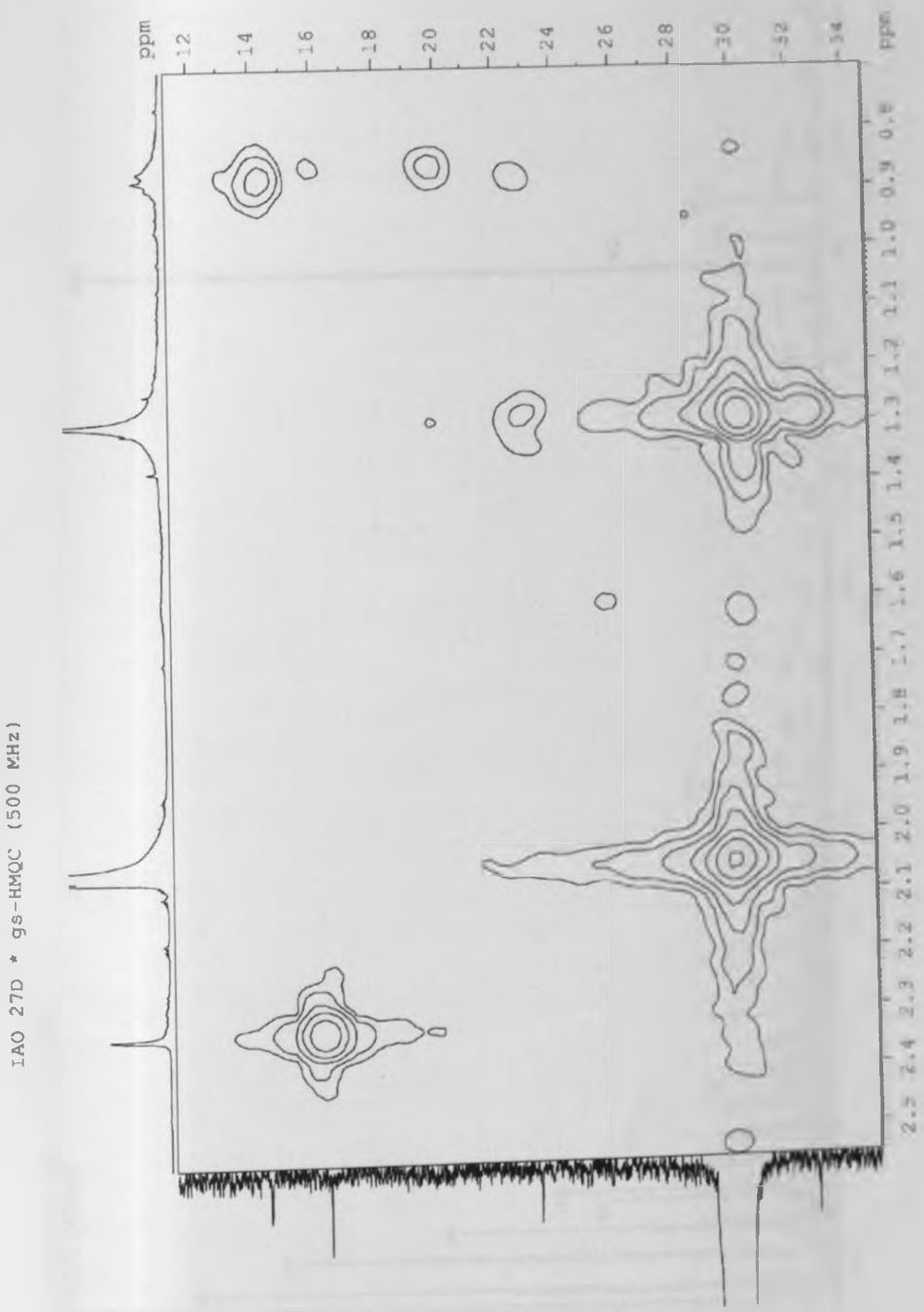
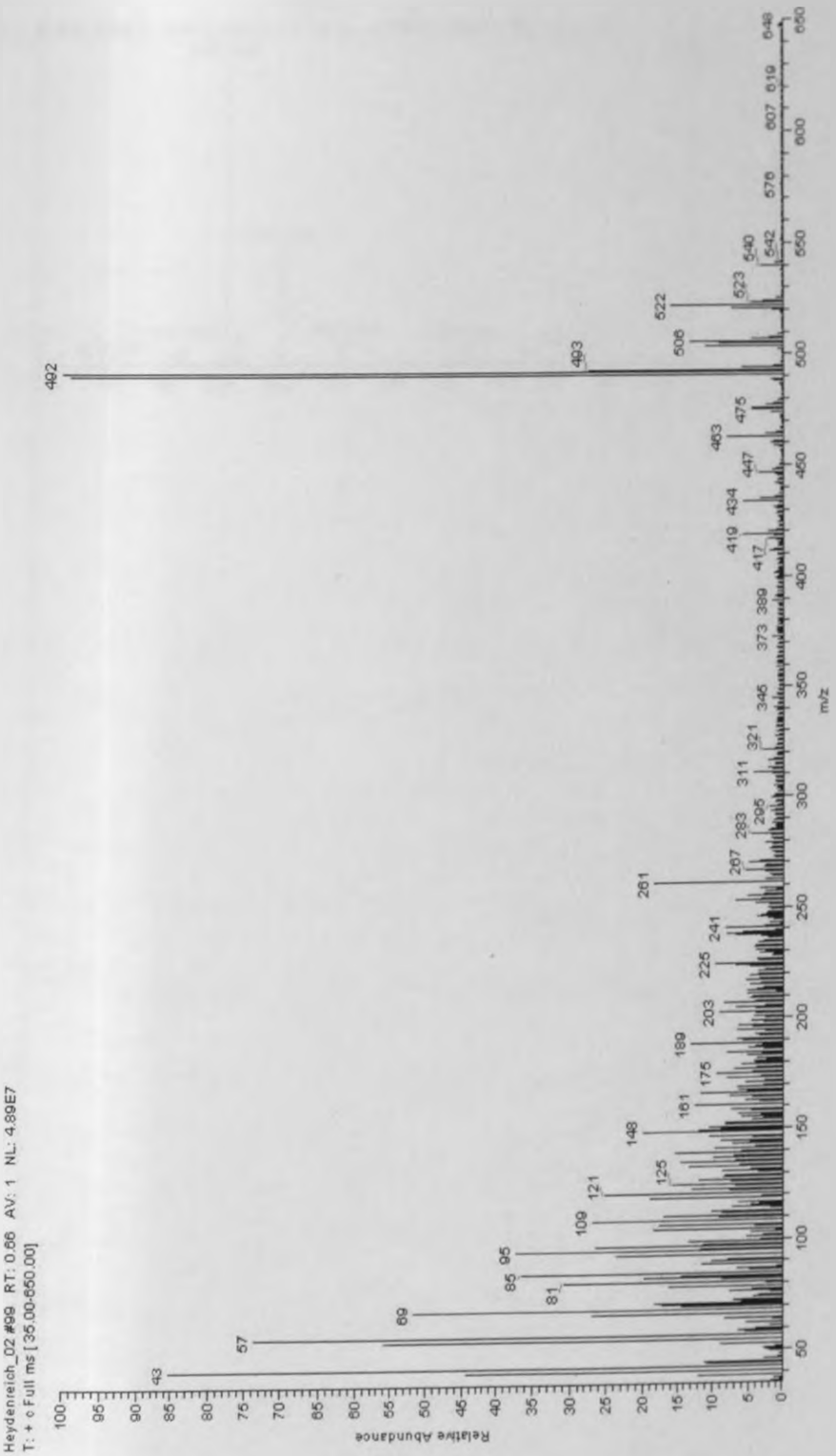


MS FOR COMPOUND 13



HRMS FOR COMPOUND 13