Isolation and Identification of Medicinal Metabolites from *Cichorium intybus*

by

Chelsea Harmon

A thesis presented to the Honors College of Middle Tennessee State University in partial fulfillment of the requirements for graduation from the University Honors College

November 2015

Isolation and Identification of Medicinal Metabolites from *Cichorium intybus*

Chelsea Harmon

APPROVED:

_____

Dr. Norma Dunlap
Department of Chemistry

_____

Dr. Greg Van Patten
Department of Chemistry

_____

Dr. Judith Iriarte-Gross
Honors Council Representative

_____

Dr. Robert Sieg
Resident Honors Scholar

Acknowledgements

I have received so much support and encouragement during the course of this project, and I am so incredibly thankful for all of those that helped me to see this goal into reality. Firstly, I would like to thank my advisor, Dr. Norma Dunlap, for allowing me to join her lab at such an early stage in my chemical education. Her vast knowledge and kind encouragements have been invaluable to me throughout my years at MTSU. I would also like to thank Matthew Wright for being my primary mentor when I was first starting in lab and for supporting my research endeavors ever since. He gave me my first true introduction to structure elucidation by NMR and introduced me to the techniques required for natural product isolation. I would like to thank all of my fellow lab mates for all of the laughs, late nights, early mornings, and support. Most especially, I would like to thank Shannon Allen for first introducing me to the idea of starting research and for being my partner in crime during the early days of our projects. I will forever cherish rocking out to 80's rock as we spent thirteen hours trying to complete our first gravity column.

I would also like to give a special thanks to my dad who sacrificed weed eating our yard in order to help me to cultivate the wild chicory that was growing, as well as sacrificing his time to help collect, wash, and prepare all of the chicory samples used during the course of this project. This project never could have happened without him. Lastly, I would like to thank all of my friends that have supported my craziness throughout this process. Most especially, I would like to thank my roommate Matthew Pyles for everything he did to calm my stress and for just being the greatest friend I could have ever asked for these past four years.

Abstract

Cichorium intybus (Chicory) has been used medicinally by a variety of cultures dating back thousands of years. Historically, it has been most commonly used as a diuretic, a hepatoprotective agent, and an anti-inflammatory agent as well as being a popular additive in coffee. Some of the compounds linked to the observed medicinal properties of chicory are β-sitosterol (wound healing), 8-deoxylactucin (anthelmintic and anti-inflammatory), and cichoric acid (anti-viral). In this project, the medicinal components of chicory were further investigated for activity against *Trypanosoma brucei*, Herpes Simplex Virus Type-I, and inflammation. Utilizing a bioassay-guided fractionation approach, two compounds, 8-deoxylactucin and lactupicrin, were isolated and identified as the active components against *T. brucei*. A third compound (11,13-dihydrolactucin) was also identified but not isolated, and thus no conclusions could be made on its activity.

Table of Contents

List of Tables v

List of Figures vi

List of Schemes vii

Chapter I: Introduction 1

Background 1

Sesquiterpene Lactones 3

Bioassay-Guided Fractionation 8

Research Focus 9

Chapter II: Materials and Methods 10

Instruments, Materials, and Reagents 10

Plant Material 11

General Extraction Procedure 11

Isolations 12

Optimized Extraction for Sesquiterpene Lactones (Gradient-Column Extraction) 18

Identification of Pure Compounds 19

Chapter III: Results and Discussion 22

Structure Elucidation of CiB3a (8-Deoxylactucin) 23

Structure Elucidation of CiB3b2 (Lactupicrin) 26

Structure Elucidation of CiB3b1 (11.13-Dihydrolactupicrin) 27

Discussion of Optimized Extraction 30

Conclusion 31

References 33

Appendices 37

Appendix A: Spectroscopy Data for CiB3a (8-Deoxylactucin) 37

Appendix B: Spectroscopy Data for CiB3b2 (Lactupicrin) 44

Appendix C: Spectroscopy Data for CiB3b1 (11,14-Dihydrolactupicrin) 51

Appendix D: Trypanosoma Brucei Assay Data 56

List of Tables

Table 1: ^{1}H and ^{13}C-NMR Data for CiB3a (8-Deoxylactucin) Compared to Literature Spectral Data **24**

Table 2: HMBC Correlations of CiB3a **26**

Table 3: ^{1}H and ^{13}C-NMR Data for CiB3b2 (Lactupicrin) Compared to Literature Spectral Data **27**

Table 4: ^{1}H and ^{13}C-NMR Data for CiB3b1 (11,13-Dihydrolactupicrin) Compared to Literature Spectral Data **29**

List of Figures

Figure 1: *Cichorium intybus* flower **1**

Figure 2: Chemical structures of known bioactive metabolites of chicory **2**

Figure 3: Structure of an isoprene unit **4**

Figure 4: Examples of sesquiterpene skeletal structures **4**

Figure 5: Examples of sesquiterpene lactones **5**

Figure 6: Structure of farnesyl pyrophosphate **5**

Figure 7: Partial mechanism explaining how Michael acceptors target exposed cysteine residues of protein **7**

Figure 8: X-ray Structure of DNA-binding domain of p65 subunit of NF-κB bound to DNA **8**

Figure 9: Schematic diagram of the general process behind bioassay-guided fractionation **9**

Figure 10: Summary of CiA fractionation **13**

Figure 11: Summary of CiB fractionation **15**

Figure 12: Summary of all fractionations **17**

Figure 13: Typical span of correlations observed in an HMBC spectrum **25**

Figure 14: 3D structure of 11,13-dihydrolactucin **28**

Figure 15: Reverse-phase (C_{18}) HPLC spectrum of CECi4b **31**

Figure 16: The 3D structures of 8-deoxylactucin and helenalin **32**

List of Schemes

Scheme 1: Biosynthesis of sesquiterpene lactones in chicory 6

CHAPTER I: INTRODUCTION

Background

Natural products have been utilized for a variety of medicinal purposes for thousands of years. Traditionally, they were used in their crude form as teas, poultices, tinctures, and other herbal concoctions. In the past hundred years, scientists have worked to isolate the active compounds within the plants, fungi, and bacteria that are responsible for the observed bioactivities of the crude forms. In 2001, it was estimated that nearly a quarter of the best-selling drugs worldwide were derived from natural products [1]. A large majority of these isolated compounds have been identified as secondary metabolites that the plant uses for its own immunologic purposes [2].

Cichorium intybus

The plant *Cichorium intybus*, commonly known as chicory, has a long history of traditional medicinal use across several cultures. It is a perennial, herbaceous plant with bright blue flowers and is of the family Asteraceae (Figure 1).

Figure 1: *Cichorium intybus* flower.

American settlers valued chicory primarily for its effects as a diuretic and laxative, but the Egyptians also believed that chicory could be used to treat tachycardia (rapid heartbeat) [3]. It is believed that chicory's calming effect on the heart is also what led to it becoming a popular additive in coffee. American settlers believed that it counteracted the nervousness caused by the caffeine in coffee. The Egyptians also used chicory leaves in a poultice to reduce inflammation. Additionally, Pakistanis used chicory as a folk remedy for liver disease [3]. Chicory has also been reported to be useful in treating leprosy, chronic fever, asthma, AIDS, and cancer [4].

Due to these reported medicinal uses, many studies have been done to attempt to identify the active components of chicory. β-Sitosterol (Figure 2A) has been identified as one of the major contributors to the wound healing potential of chicory poultices used in Turkey [5].

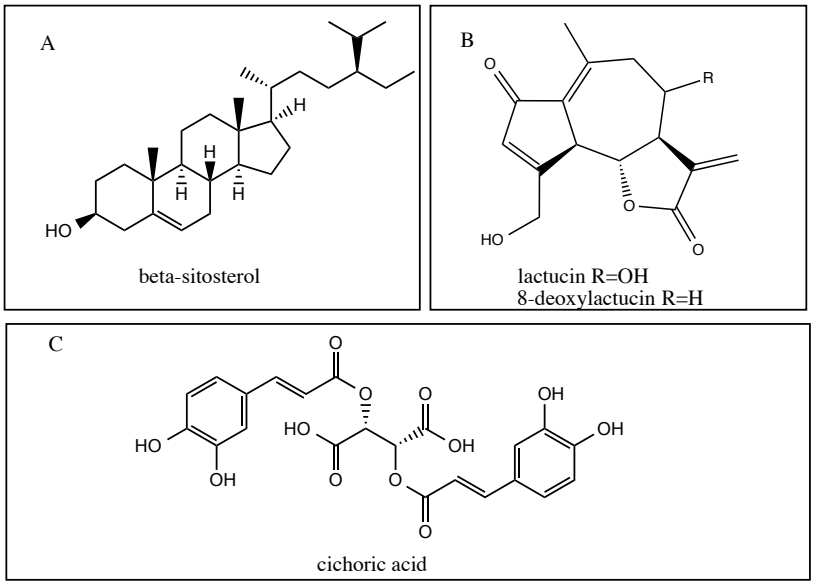


Figure 2: Chemical structures of known bioactive metabolites of chicory: (A) β-sitosterol (B) Sesquiterpene lactones: lactucin and 8-deoxylactucin (C) Cichoric acid.

This is due to the compound's significant anti-inflammatory and anti-oxidant properties as well as its inhibitory effect against hyaluronidase and collagenase (enzymes that are degenerative to structural proteins involved in cell knitting) [5]. Several active sesquiterpene lactones have also been isolated. The most significant sesquiterpene lactones are lactucin (Figure 2B), which has exhibited significant activity against malaria [6], and 8-deoxylactucin, which has exhibited significant anthelmintic and antifungal activities [7, 8]. Anti-trypanosome activity has also been reported in chicory, but the active compound(s) have not yet been isolated [9]. A broad range of chicory metabolites have been found to be active against several bacterial pathogens including *Staphylococcus aureus*, *Micrococcus luteus*, and *Pseudomonas aeruginosa* [4]. Specific compounds have not been linked to many of these antimicrobial activities. Cichoric acid (Figure 1C) has also been identified as a significant antiviral agent. It is active against hepatitis B virus (HBV), human immunodeficiency virus (HIV), and herpes simplex virus (HSV) [10]. Chicory is also a rich source of inulin, a β-(2-1)-fructose polymer, which is currently being investigated for its anti-diabetic properties [11].

Sesquiterpene Lactones

Definition

Terpenes are a class of biological metabolites that are defined by containing a combination of a repetitive structural unit known as an isoprene unit. Sesquiterpenes are terpenes consisting of three isoprene units (Figure 3).

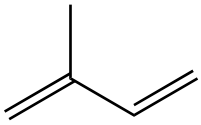


Figure 3: Structure of an isoprene unit

These isoprene units can be organized in a multitude of arrangements, which makes sesquiterpenes a structurally diverse compound class. Some of the major sesquiterpene skeletal arrangements that occur in plants are germacranes, eudesmanes, and guaianes (Figure 4) [12].

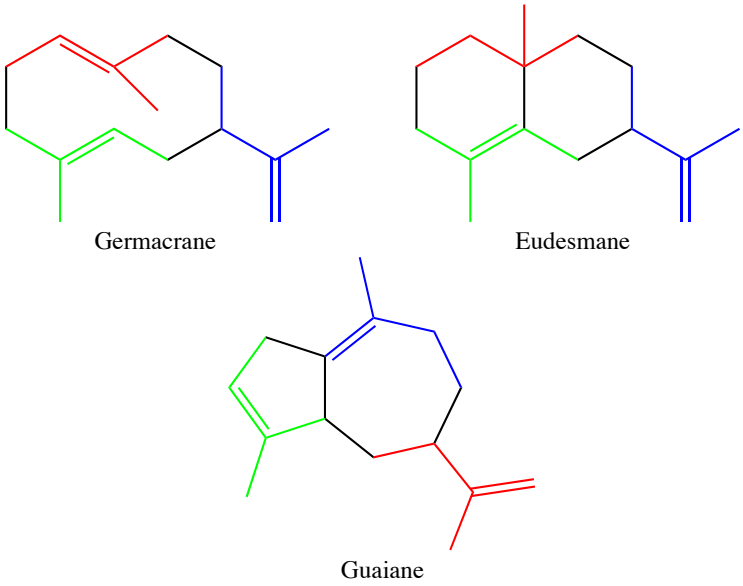


Figure 4: Examples of sesquiterpene skeletal structures. The individual isoprene units have been color-coded [12].

A sesquiterpene lactone is a sesquiterpene that has been enzymatically oxidized. Often times, this oxidation results in a lactone ring closure. Examples of sesquiterpene lactones from each of the main skeletal structures include costunolide, telekine, and lactucin (Figure 5) [13].

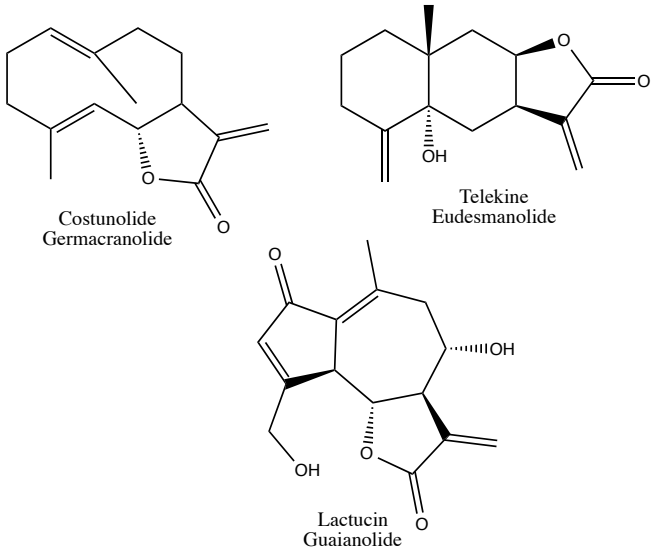


Figure 5: Examples of sesquiterpene lactones [13].

Biosynthesis in Chicory

Because sesquiterpene lactones consist of three isoprene units, their biosyntheses generally all begin with farnesyl pyrophosphate (FPP) (Figure 6), which is the simplest linear sesquiterpene in an activated form.

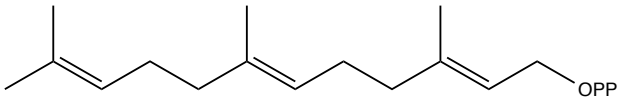
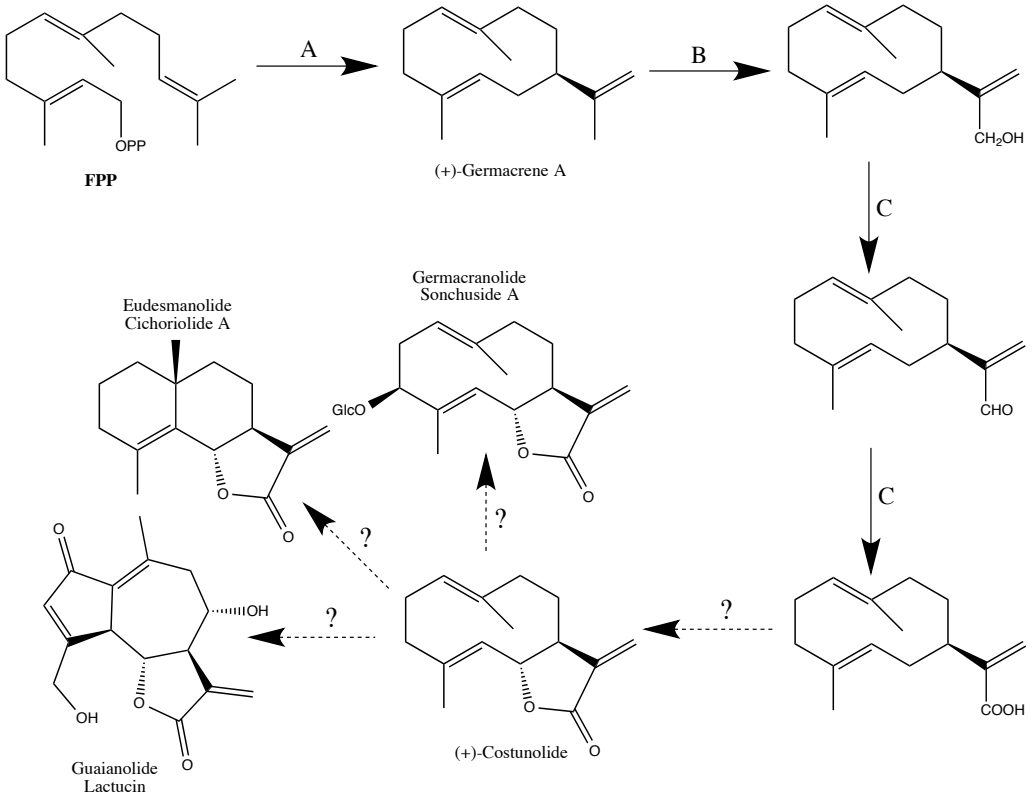


Figure 6: Structure of farnesyl pyrophosphate.

Sesquiterpene lactones are one of the most abundant metabolites present in chicory. They are bitter compounds and play a large role in the plant's anti-foraging defense mechanism. When the plant is damaged, it releases jasmonic acid, which in turn upregulates farnesyl pyrophosphate synthase [14]. The farnesyl pyrophosphate is then cyclized to (+)-germacrene A by germacrene synthase. The (+)-germacrene A is then oxidized in a series of NADPH/$NADP^+$-dependent steps catalyzed by a cytochrome P450 hydroxylase and $NADP^+$-dependent dehydrogenases. It is then

hypothesized that the resulting compound is hydroxylated at the C_6-position in order to complete the lactone formation of (+)-costunolide, though this still has yet to be proven. It is suspected that all germacranolides, eudesmanolides, and guaianolides are derived from costunolide, though the exact enzymatic processes that connect them are still unknown (Scheme 1) [12].



Scheme 1: Biosynthesis of sesquiterpene lactones in chicory. A: Germacrene Synthase, Mg^{2+}, B: NADPH, O_2, Cytochrome P450 hydroxylase, C: $NADP^+$-dependent dehydrogenases [12].

Known Medicinal Mechanisms of Action

Sesquiterpene lactones exhibit a wide range of biological activities. They have been linked to antimicrobial, anti-inflammatory, antitumor, and anti-malarial activities [15, 16, 17, 18]. Most of these activities are linked to α, β-unsaturated

carbonyls that act as Michael acceptors and react readily with nucleophiles such as the sulfhydryl group on cysteine residues [19] (Figure 7).

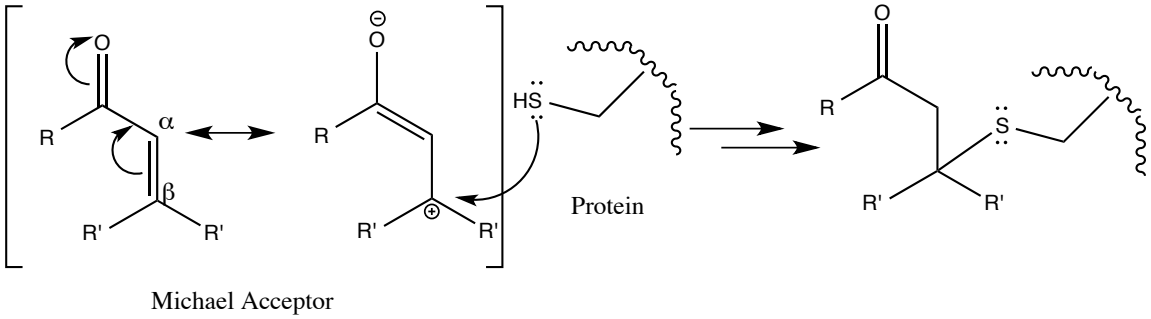


Figure 7: Partial mechanism explaining how Michael acceptors target exposed cysteine residues of protein.

One of the most well investigated activities exhibited by sesquiterpene lactones is their anti-inflammatory effect. Studies have shown that sesquiterpene lactones, including 8-deoxylactucin, tend to inhibit the transcription factor NF-κB. This transcription factor is responsible for activating genes for cyclooxygenase-II (COX-II) among other proteins involved in immune responses and cell adhesion. COX-I and II are generally the targets for anti-inflammatory agents due to their role in producing the prostaglandins directly responsible for triggering an inflammatory response. Extensive studies involving X-ray crystallography analysis of NF-κB and the sesquiterpene lactone helenalin have shown that helenalin cross-links two cysteine residues in the DNA-binding domain of p65 subunit of NF-κB as seen in Figure 8 [19].

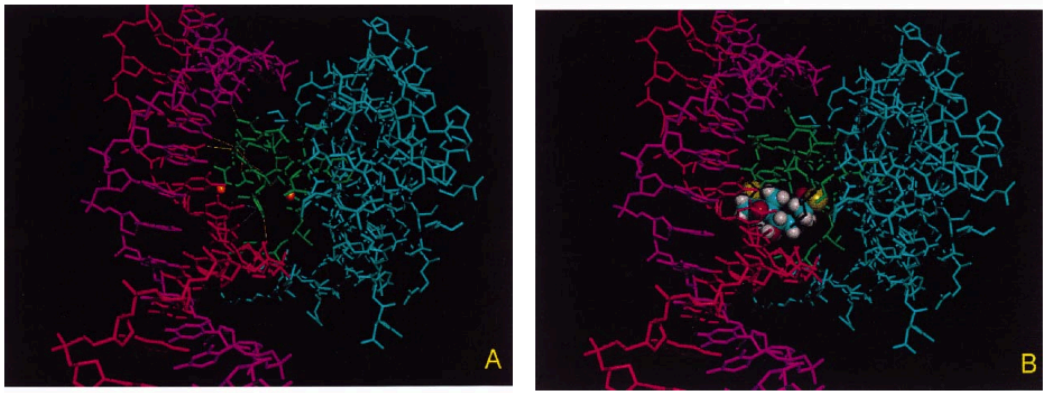


Figure 8: A) X-ray structure of DNA-binding domain of p65 subunit of NF-κB bound to DNA. Key sulfhydryl groups represented as red balls. B) X-ray structure of helenalin cross-linking the two sulfhydryl groups and preventing proper binding to DNA. Figure used with permission of the Journal of Medicinal and Bioorganic Chemistry [19].

Bioassay-Guided Fractionation

Bioassay-guided fractionation is one of the oldest strategies used in drug discovery. It's an intuitive process in which the sample in question is divided into fractions, assays are performed to determine which fraction holds the active compound, and the process repeats until a single active compound is identified. To obtain a sample capable of being fractionated, the plant (or other natural source) is dried and pulverized to a powder that is then suspended in solvent and allowed to stir over low heat. The resulting solution is then decanted, evaporated down, and partitioned based on solubility using a separatory funnel. The active partition is then carried forward and fractionated through various methods of column chromatography. This process is summarized in Figure 9.

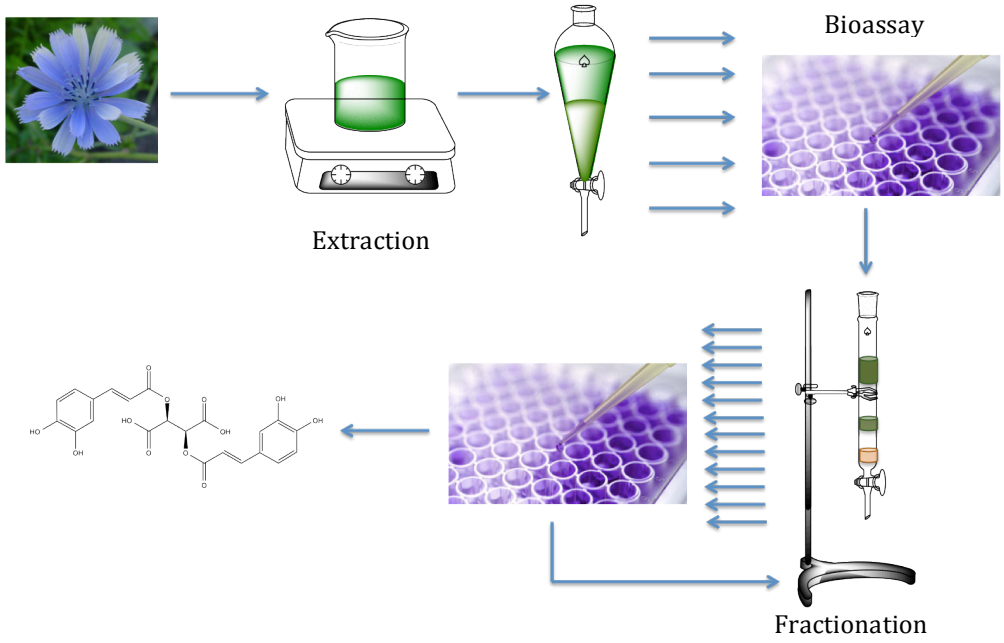


Figure 9: Schematic diagram of the general process behind bioassay-guided fractionation.

Research Focus

Chicory presents a wide range of biological activity, however, the exact compounds responsible for some of chicory's medicinal properties are yet to be determined. At MTSU, the crude extracts of chicory have exhibited anti-HSV, antifungal, anti-inflammatory, and anti-trypanosome activities. The anti-HSV activity is located in a partition of the crude extract that, theoretically, should not contain cichoric acid. The anti-trypanosome activity of chicory has been previously documented, but there is no literature that identifies the compound responsible for the activity. The antifungal activity is present in many partitions and may yield multiple compound identifications. The compounds isolated from following these activities through a bioassay-guided fractionation are presented here.

CHAPTER II: MATERIALS AND METHODS

Instruments, Materials, and Reagents

NMR data obtained using a 500MHz FT-NMR model ECA-500 JEOL (Peabody, MA) purchased with funding provided by the National Science Foundation through the NSF-MRI program (#0321211). Chemical shifts are reported in parts per million (ppm) in reference to tetramethylsilane (TMS). Splitting patterns are represented by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets), td (triplet of doublets), ddq (doublet of doublet of quartets), and ddd (doublet of doublet of doublets). Coupling constants (J values) are recorded in Hz. High-resolution electrospray ionization-mass spectrometry (ESI-MS) was performed at Notre Dame University, Notre Dame, Indiana.

Thin-layer chromatography (TLC) was performed on glass plates coated with silica gel with UV active backing purchased from Fisher Scientific, Pittsburgh, PA. TLC plates were analyzed utilizing UV light (254 nm) absorbance and subsequent staining with either phosphomolybdic acid (PMA) or anisaldehyde (reagent grade, Aldrich, Milwaukee, WI) stain solutions. Gravity column chromatography was performed with silica gel, 63-200 micron, 70-230 mesh ASTM (reagent grade, Fisher Scientific, Pittsburgh, PA). Flash column chromatography was performed with silica gel, 32-63 micron ASTM (reagent grade, Fisher Scientific, Pittsburgh, PA), and, where indicated, flash column chromatography was also performed on an ISCO CombiFlash R_f 200 Teledyne ISCO, (Lincoln, NE) using a Teledyne ISCO cartridge preloaded with 5g of normal phase silica and a Teledyne ISCO preloaded 12g flash

column. All HPLC analysis was performed on an Ultimate 3000 Dionex with a photodiode array detector and an autosampler using a C_{18} column and acetonitrile/water gradient.

Dichloromethane, methanol, acetone, ethyl acetate, ethanol, and hexanes were purchased from Fisher Scientific, Pittsburgh, PA. Chloroform was purchased Aldrich, Milwaukee, WI. Deutero-acetone (Acetone-d_6) were purchased from Aldrich, Milwaukee, WI.

Plant Material

Aerial leaves and flowers of *Cichorium intybus* were collected fresh from a field in Johnson City, TN (36°15'48"N, 82°24'6"W) in October of 2014. A second batch was collected in June of 2015.

Naming Scheme

Fractions named by the abbreviation "Ci" with a series of letters and numbers depicting the degree of fractionation. The first degree of fractionation is depicted by a capital letter, the second degree of fractionation is depicted by a number, the third degree of fractionation is depicted by a lower case letter, and the fourth degree of fractionation is depicted by a number.

General Extraction Procedure

The leaves and flowers were washed thoroughly with water and frozen for transport. They were then ground with pestle while suspended in liquid nitrogen. The ground, frozen plant material was then lyophilized to remove the water. The dried material (67.08 g) was macerated overnight in methanol at 40 °C while stirring. The methanol was decanted after approximately 24 hours and the

maceration was repeated for a total of three times. The decanted methanol was filtered and evaporated using a rotary evaporator at 40 °C yielding the initial crude extract (11.85 g, 17.7%). A portion of the methanol extract (Ci: 9.46 g) was then partitioned between hexanes and water to give the hexanes partition (CiA: 4.60 g [48.6%]) and an aqueous partition. The aqueous partition was extracted with chloroform to give the chloroform partition (CiB: 495.4 mg [5.2%]) and an aqueous partition. This aqueous partition was extracted with ethyl acetate to give the ethyl acetate partition (CiC: 470.5 mg [5.0%]) and an aqueous partition. Finally, the aqueous partition was extracted with butanol to give the butanol partition (CiD: n/a*) and the final aqueous partition (CiE: 742.1 mg [18.0%]).

*Note: A weight was not obtained for CiD because of the difficulties met with drying the sample. Since it was not found to be active during the initial testing stages, the sample was discarded.

Isolations

Fractionation of Hexanes Partition (CiA): CiA exhibited >95% inhibition against HSV-I [20]. CiA was fractionated by gravity column chromatography of CiA (1.86 g) on silica gel (63-200 μm) with a step gradient of hexanes (Hex) and increasing amounts of ethyl acetate (EA) (100% Hex, 85:15, 70:30, 55:45, 40:60, 25:75, 100% EA, 100% methanol [flush]; 200 mL each) yielded eight fractions (CiA1) 883.5 mg [47.5%], (CiA2) 320.6 mg [17.2%], (CiA3) 372.1 mg [20.0%], (CiA4) 86.8 mg [4.7%], (CiA5) 136.9 mg [7.4%], (CiA6) 74.1 mg [4.0%], **(CiA7) 52.2 mg [2.8%]**, (CiA8) 200.7 mg [10.8%]). CiA7 was the sole fraction to retain bioactivity. Thin-layer chromatography (TLC) on silica gel, eluting with a 1:1 mixture of

dichloromethane and acetone, revealed five major spots when stained with phosphomolybdic acid (PMA). Flash chromatography of CiA7 (52.2 mg) on silica gel (32-63 μm) with a step gradient of dichloromethane (DCM) with increasing acetone (DCM: acetone; 4:1, 7:3, 1:1, 100% methanol [flush]; volumes based on appearance and disappearance of spots) yielded five fractions with each of the major spots isolated (CiA7a) 8.8 mg, **(CiA7b) 2.1 mg**, (CiA7c) 2.5 mg, (CiA7d) 10.8 mg, **(CiA7e) 10.5 mg)**. CiA7b and CiA7e retained the bioactivity with CiA7e (MeOH flush) exhibiting the greatest inhibition. A summary of the fractionation of CiA is presented in Figure 10.

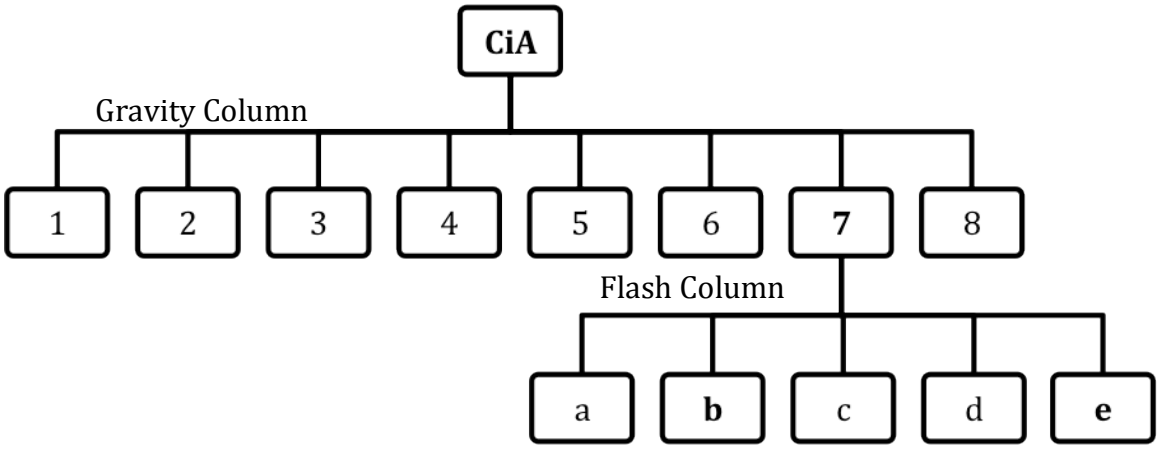


Figure 10: Summary of CiA fractionation. Active fractions have been bolded.

Fractionation of Chloroform Partition (CiB): CiB exhibited >95% inhibition against *Trypanosoma brucei* [21]. TLC on silica gel eluting with 1:1 DCM/acetone revealed six spots. CiB was fractionated by flash column chromatography of CiB (281.8 mg) on silica gel (32-63 μm) with a step gradient of DCM with increasing acetone (100% DCM, 5:95, 90:10, 85:15, 80:20, 75:25, 70:30, 60:40, 50:50, 40:60, 30:70, 100% methanol; 100 mL each) yielded five combined

fractions: (CiB1) 33.5 mg, (CiB2) 41.9 mg, **(CiB3) 85.4 mg**, (CiB4) 43.3 mg, (CiB5) 98.2 mg. CiB3 was the only fraction that contained a single distinct spot. Fortuitously, CiB3 was the primary fraction to retain bioactivity (86.00% Growth Inhibition) [21]. ^{1}H-NMR revealed that CiB3 consisted primarily of 2-3 compounds with similar skeletal structures. Through further TLC analysis on silica gel eluting with 4:1 ethyl acetate/hexane, three major spots were observed.

In a subsequent flash column, another batch of CiB (360 mg) was fractionated using the same step gradient as described above. Only fractions suspected to contain the three compounds observed in CiB3 were collected. These fractions were combined to give two new fractions: CiB3a (67.0 mg, 18.6%) and CiB3b (28.1 mg, 7.8%).

CiB3a was found to be an isolated compound and identified by NMR analysis as 8-deoxylactucin. This compound was identified as the most potent component of CiB3 against *T. brucei* [21].

CiB3b was found to contain a compound that crystallized out of acetone. A vial of CiB3b was suspended in enough acetone to fully dissolve the sample and then the acetone was slowly evaporated by air until crystals began to form. The vial was then allowed to sit in the freezer so that the crystals could continue to grow. The acetone was then pipetted into a separate tared vial. The crystals were rinsed with three drops of acetone three times. The acetone soluble portion (CiB3b1) was found to be in a 2:1 ratio with the crystallized compound (CiB3b2) according to ^{1}H-NMR integrations. CiB3b2 was identified through NMR as lactupicrin [22]. The major compound in CiB3b1 was identified through NMR as 11,13-dihydrolactupicrin [22].

8-deoxylactucin (CiB3a) exhibited the most potent activity of the three isolated compounds. A summary of the fractionation of CiB is presented in Figure 11.

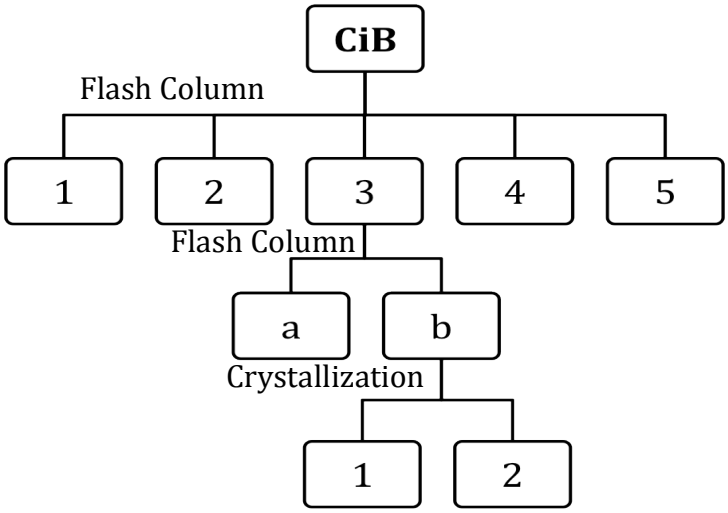


Figure 11: Summary of CiB Fractionation.

Fractionation of Ethyl Acetate Partition (CiC): CiC exhibited promising activity as an anti-inflammatory agent [23]. Interest in this activity increased once 8-deoxylactucin (a known anti-inflammatory agent) was identified in partition CiB. Components of CiC were found to precipitate rapidly out of DCM, and an attempt was made to separate by trituration. TLC on silica gel eluting with 4:1 EA/Hex revealed that the DCM soluble partition contained only chlorophylls. The precipitate was resuspended in acetone and analyzed by TLC, which revealed five discrete spots. This suspension was then loaded on a 5g RediSepRf normal-phase silica loading cartridge, and a flash column was performed on the CombiFlashRf utilizing a steady gradient increasing from 0%-100% EA/Hex in 20 min and maintaining 100% EA until all substances were extracted from column to afford six combined fractions

(CiC1) 6.0 mg, (CiC2) 25.6 mg, (CiC3) 61.7 mg, (CiC4) 69.9 mg, (CiC5) 21.6 mg, (CiC6) 257.2 mg. No further purification was done on these fractions.

Note: Some recorded weights of the fractions mentioned above may be higher than the true value due to hygroscopic and oil-like properties that prevented complete evaporation of solvents.

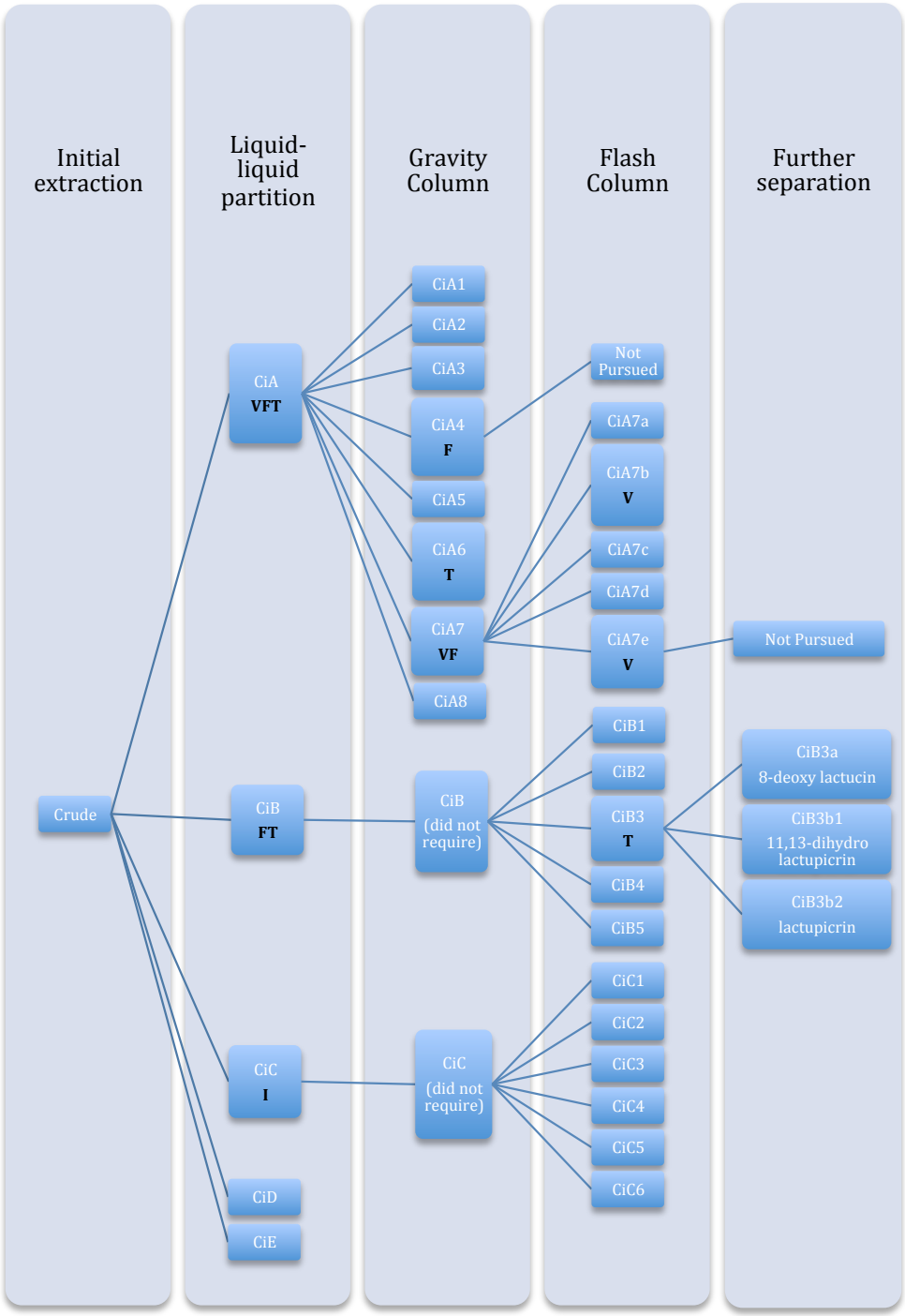


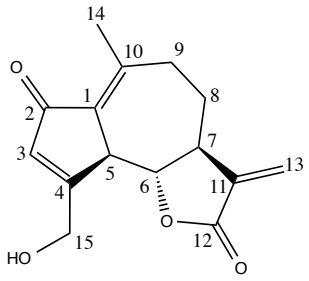
Figure 12: Summary of all fractionations. (**V**: Anti-HSV, **F**: Anti-Fungal, **T**: Anti-Trypanome, **I**: Anti-Inflammatory)

Optimized Extraction for Sesquiterpene Lactones (Gradient-Column Extraction)

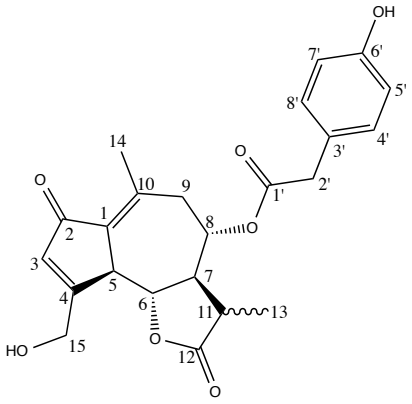
The raw plant material was prepared as described for the general extraction. This dried plant material was then pulverized to a fine powder in a coffee blender. The powdered plant material (5 g) was then mixed with 1 g of celite to prevent clumping. A 2 cm column was then plugged with cotton and filled with the powdered plant material. Another cotton plug was placed on top of the powdered plant material and the plant material was packed down as tightly as possible. The celite-dispersed material was eluted successively with 100% hexanes (100 mL); 1:1 hex/acetone (100 mL); 100% acetone (100 mL); 1:1 acetone/MeOH (100 mL); and 100% MeOH (100 mL) to yield five column extraction (CE) fractions: (CECi1) 176.5 mg, (CECi2) 83.6 mg, (CECi3) 33.2 mg, (CECi4) 278.2 mg, (CECi5) 252.3 mg for a total of 823.8 mg (16.5%) of filtered extract. TLC analysis showed that 8-deoxylactucin and lactupicrin were confined to CECi4. A liquid-liquid extraction was performed on 278 mg CECi4 as described in the general extraction to yield 85.0 (30.6%) in hexanes (CECi4A) and 14.0 mg (5.0%) in chloroform (CECi4B).

A general maceration extraction (ME) was performed on 5 g of powdered plant material in 1:1 acetone/MeOH for comparison, which yielded 343.8 mg (6.9%) (MECi4) and 17.7 mg (5.1%) in the chloroform partition (MECi4B). Reverse-Phase (C_{18}) HPLC analysis was conducted on both CECi4b and MECi4b. Both fractions showed a high purity 1:1 mixture of 8-deoxylactucin and lactupicrin.

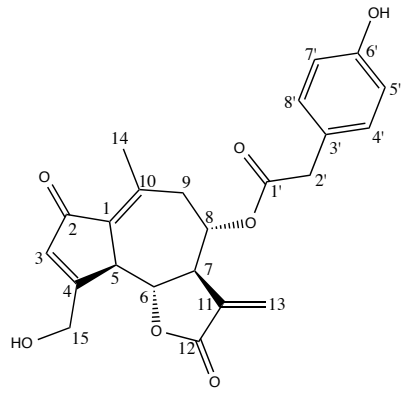
Identification of Pure Compounds



8-Deoxylactucin (CiB3a): The following spectroscopic data was obtained in acetone-d_6: ^{1}H-NMR, ^{13}C-NMR, DEPT$_{135}$, COSY, HMQC, and HMBC. ^{1}H-NMR (acetone d_6, 500 MHz): δ6.34 (dd, J=3.1, 1.7 Hz, 1H, H3), 6.01 (d, J=3.4 Hz, 1H, H13a), 5.52 (d, 3.2 Hz, 1H, H13b), 4.90-4.75 (m, 1H, H15a), 4.46-4.31 (m, 1H, H15b), 3.78 (d, J=10.2 Hz, 1H, H5), 3.63 (t, J=10.1 Hz, 1H, H6), 3.03(ddq, J= 11.4, 9.7, 3.1 Hz, 1H, H7), 2.60 (t, J=12.8 Hz, 1H, H9a), 2.38 (s, 3H, H14), 2.35 (ddd, J=7.6, 5.4, 1.5 Hz, 1H, H9b), 2.02 (dt, J=4.4, 2.2 Hz, 1H, H8b), 1.43 (td, J=12.9, 1.6, 1H, H8a). ^{13}C-NMR (Acetone D_6, 125 MHz): δ194.6 (C2), 173.8 (C12), 168.5 (C4), 152.3 (C10), 139.7 (C1), 132.2 (C3), 131.6 (C11), 117.6 (C13), 84.0 (C6), 62.0 (C15), 52.3 (C7), 49.8 (C5), 36.8 (C9), 24.2 (C8), 20.7 (C14). MS (ESI), *m/z* ($C_{15}H_{17}O_4$) calculated for 261.1121, found 261.1116 ($M^+ + H$).



11,13-Dihydrolactupicrin (CiB3b1): The following spectroscopic data was obtained in acetone-d_6: ^{1}H-NMR and COSY. ^{1}H-NMR (acetone-d_6, 500 MHz): δ8.34 (s, 1H, C6'-OH), 7.12 (d, J=8.5 Hz, 2H, H4'/8'), 6.77 (d, J=8.5 Hz, 2H, H5'/7'), 6.35 (d, J=1.2 Hz, 1H, H3), 4.83 (td, J=10.6, 1.9 Hz, 1H, H8), 4.82 (t, J=17.7, 1H, H15a), 4.37 (t, J=18.5, 1H, H15b), 3.85 (m, 1H, H6), 3.73 (d, J= 10.2 Hz, 1H, H5), 3.60 (s, 2H, H2'), 3.48 (m, 1H, H7), 2.84 (m, 1H, H9a), 2.34 (m, 1H, H9b), 2.35 (s, 3H, H14), 1.17(s, 3H, H13).



Lactupicrin (CiB3b2): The following spectroscopic data was obtained in Acetone-D_6: ^{1}H-NMR, ^{13}C-NMR, DEPT$_{135}$, COSY, HMQC, and HMBC. ^{1}H-NMR (Acetone D_6, 500 MHz): δ8.32 (s, 1H, C6'-OH), 7.15 (d, J=8.4 Hz, 2H, H4'/8'), 6.79 (d, 8.5 Hz, 2H, H5'/7'), 6.36 (s, 1H, H3), 5.89 (d, J=3.2 Hz, 1H, H13a), 5.38 (d, J=3.2 Hz, 1H, H13b),

4.91 (td, J=10.5, 2.0 Hz, 1H, H8), 4.84 (m, 1H, H15a), 4.38 (m, 1H, H15b), 3.86(d, J= 6.5 Hz, 1H, H5), 3.85 (dd, J=11.8, 6.2 Hz, 1H, H6), 3.62 (s, 2H, H2'), 3.48 (m, 1H, H7), 2.84 (m, 1H, H9a), 2.40 (dd, J=13.5, 2.0, 1H, H9b), 2.35 (s, 3H, H14). ^{13}C-NMR (Acetone D_6, 125 MHz): δ193.94 (C2), 173.92 (C4), 170.62 (C1'), 167.81 (C12), 156.70 (C6'), 144.57 (C10), 137.01 (C11), 133.43 (C1), 132.42 (C3), 130.54 (C4'/8'), 124.67 (3'), 120.54 (C13), 115.40 (C5'/7'), 80.86 (C6), 69.62 (C8), 62.01 (C15), 54.14 (C7), 48.26 (C5), 43.72 (C9), 40.14 (C2'), 20.11 (C14). MS (ESI), *m/z* ($C_{23}H_{22}O_7$) calculated for 411.1399, found 411.1410 (M^+ + H).

CHAPTER III: RESULTS AND DISCUSSION

Through the course of this research, crude plant material from *C. intybus* was purified using bioassay-guided fractionation. Two compounds, 8-deoxylactucin and lactupicrin, were isolated. 11,13-Dihydrolactupicrin was isolated in a 2:1 ratio with lactupicrin, and was identified through comparison with literature NMR data as well as examining the COSY spectra of the compound. 8-Deoxylactucin and lactupicrin both exhibited activity against *Trypanosoma brucei* with 8-deoxylactucin being the most potent with >90% inhibition at 0.390 μg/mL and an IC_{50} of 0.16±0.05 μg/mL.

The bulk of the extractions performed for this project were maceration extractions. This type of extraction requires three days of steeping and decanting the plant material in methanol. The decanted solution must then be thoroughly filtered in order to ensure the removal of small insoluble particles. This solution must then be dried through rotary evaporation. While the maceration extractions were efficient in the volume of material extracted, they were not very time efficient. The gradient-column extraction was developed to optimize the extraction of the isolated sesquiterpene lactones. This extraction method was inspired by an extraction procedure developed by Nancy Dewi Yuliana from Leiden University in the Netherlands in which she packed an emptied HPLC column and performed a similar extraction to prepare samples for metabolomic-type studies [24]. The gradient-column extraction took about an hour to complete from the time the column was loaded to when the final fraction was evaporated to yield a pre-filtered and fractionated extract. This was a great improvement from the four day process required to reach the same point using the general maceration extraction method.

Structure Elucidation of CiB3a (8-Deoxylactucin)

Initial analysis of the ^{1}H-NMR data indicated a terminal alkene with two nonequivalent hydrogens (6.01-5.52 ppm, 2H) and a suspected CH_2 adjacent to an oxygen with two nonequivalent hydrogens (4.90-4.31 ppm, 2H). This observation was supported by the HMQC 2D spectra, which revealed the presence of four nonequivalent CH_2 groups having eight nonequivalent hydrogens. Analysis of the ^{13}C-NMR indicated the presence of three potential carbonyl groups (194.6-168.5 ppm); however, the peak at 168.5 ppm was later determined to be the β carbon of an αβ-unsaturated ketone, which is known to shift farther downfield than expected for an alkene carbon.

With these observations in place, the known structural fragments were linked to those typical of a guaianolide sesquiterpene lactone. Utilizing the HMQC spectra, the ^{1}H and ^{13}C peaks were correlated to each other and then compared to literature data of sesquiterpene lactones previously isolated from chicory. From this comparison, 8-deoxylactucin was identified as the closest match to the fraction labeled CiB3a. The slight differences in peak values are due to differences in deuterated solvents used to obtain data (Table 1). *Note: Fraction CiB3a was not very soluble in CD_3OD or $CDCl_3$.*

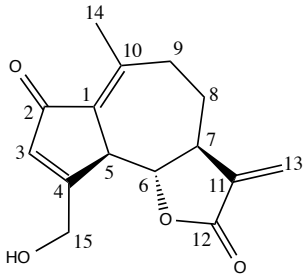


Table 1: ^{1}H and ^{13}C-NMR Data for CiB3a (8-Deoxylactucin) compared to literature spectral data				
Position	**Literature [22] CD_3OD**		**Experimental Data Acetone-D_6**	
	δ ^{1}H-NMR (mult, *J* Hz)	δ ^{13}C-NMR (type)	δ ^{1}H-NMR (mult, *J* Hz)	δ ^{13}C-NMR (type)
1	---	140.7* (q)	---	131.6 (q)
2	---	195.7 (q)	---	194.6 (q)
3	6.41 (d, 1.5)	133.2 (CH)	6.34 (dd, 3.1, 1.7)	132.2 (CH)
4	---	170.4** (q)	---	173.8 (q)
5	3.82 (d, 10.1)	51.2 (CH)	3.78 (d, 10.2)	49.8 (CH)
6	3.64 (dd, 10.1, 9.8)	85.6 (CH)	3.63 (t, 10.1)	84.0 (CH)
7	3.03 (ddddd, 10.0, 9.8, 3.7, 3.1, 1.8)	53.6 (CH)	3.03 (ddq, 11.4, 9.7, 3.1)	52.3 (CH)
8	8α: 2.26 (dddd, 13.4, 1.8, 1.8, 1.2) 8β: 1.44 (dddd, 13.4, 10, 9.8, 1.9)	38.2 (CH_2)	8α: 2.02 (dt, 4.4, 2.2) 8β: 1.43 (td, 12.9, 1.6)	24.2 (CH_2)
9	9α: 2.43 (dd) 9β: 2.36 (ddd)	25.3 (CH_2)	9α: 2.60 (t, 12.8) 9β: 2.35 (ddd, 7.6, 5.4, 1.5)	36.8 (CH_2)
10	---	155.8 (q)	---	152.3 (q)
11	---	133.1* (q)	---	139.7 (q)
12	---	176.2** (q)	---	168.5 (q)
13	13: 6.09 (d, 3.7) 13': 5.56 (d, 3.1)	119.2 (CH_2)	13: 6.01 (d, 3.4) 13': 5.52 (d, 3.2)	117.6 (CH_2)
14	2.43 (s)	22.0 (CH_3)	2.38 (s)	20.7 (CH_3)
15	15: 4.88 (dd, 18.3, 1.5) 15': 4.42 (d, 18.3)	63.1 (CH_2)	15: 4.90-4.75 (m) 15': 4.46-4.31 (m)	62.0 (CH_2)

*Based on HMBC, these two peak assignments are incorrect and should be switched.
**Based on HMBC, These Two peak assignments are incorrect and should be switched.

The HMBC spectrum was then analyzed to observe multiple-bond correlations in order to verify the position assignments of each peak. This technique correlates protons to carbons separated by two and three bonds or four bonds if one of the bonds is a pi-bond (Figure 13).

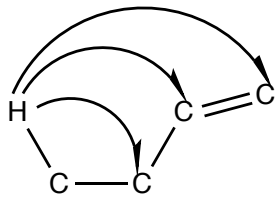


Figure 13: Typical span of correlations observed in an HMBC spectrum.

As seen in Table 1, the literature correlated C1 to the ^{13}C peak at 140.7 ppm and C11 to the peak at 133.1 ppm, but this assignment is not in agreement with what was observed in the HMBC spectrum of CiB3a. H7 was corresponding to the carbon peak at 139.7 ppm and H14 was corresponding to the carbon peak at 131.6 ppm. Based on the literature, the peak at 139.7 would correspond to C1 and the peak at 131.6 ppm would correspond to C11. C1 is not within three bonds of H7 and C11 is not within three bond lengths of H14. However, C11 is only two bonds away from H7 and C1 is only two bonds away from H14. For this reason, the peak values of C1 and C11 were switched compared to what the literature claimed. The peak values of C4 and C12 were switched for the same reason based on HMBC correlations with H3 and H13 respectively. The observed HMBC correlations are displayed in Table 2.

Table 2: HMBC Correlations of CiB3a	
Proton Position	**Correlated Carbon**
H3	C5, C2, C15, C1, C4
H5	C7, C6, C1, C4, C3, C10, C2
H6	C8, C5, C1
H7	C11, C6
H8	C9, C7, C6, C10
H9	C14, C8, C7, C1, C10, C2
H13	C7, C12
H14	C2, C9, C1, C10, C6
H15	C4, C3

Structure elucidation of CiB3b2 (Lactupicrin)

Initial analysis of the ^{1}H-NMR spectrum revealed the presence of many similar peaks to those found in CiB3a (8-deoxylactucin). The main difference was the presence of two doublets in the aromatic region characteristic of a para-substituted aryl-ring (7.15-6.79 ppm, 4H) and the presence of a broad singlet at 8.32 ppm characteristic of an aryl alcohol. Upon comparison to the values presented in the literature, CiB3b2 was easily identified as lactupicrin (Table 3). *Note: Fraction CiB3b2 was not very soluble in CD_3OD or $CDCl_3$.*

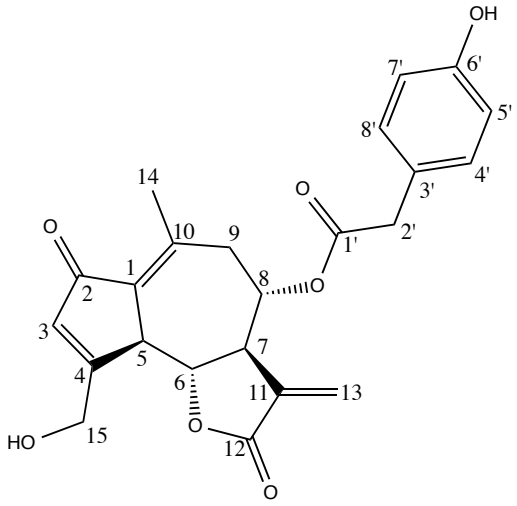


Table 3: ^{1}H and ^{13}C-NMR Data for CiB3b2 (Lactupicrin) compared to literature spectral data				
Position	**Literature [22] CD_3OD**		**Experimental Data Acetone-D_6**	
	δ ^{1}H-NMR (mult, *J* Hz)	δ ^{13}C-NMR (type)	δ ^{1}H-NMR (mult, *J* Hz)	δ ^{13}C-NMR (type)
1	---	134.6 (q)	---	133.43 (q)
2	---	197.0 (q)	---	193.94 (q)
3	6.40 (dd, 1.2, 0.8)	133.6 (CH)	6.36 (s, 1H)	132.42 (CH)
4	---	176.3 (q)	---	173.92 (q)
5	3.83 (bd, 10.0)	49.8 (CH)	3.86 (d, 6.5, 1H)	48.26 (CH)
6	3.76 (dd, 10.0, 9.9)	82.6 (CH)	3.84 (dd, 11.8, 6.2, 1H)	80.86 (CH)
7	3.41 (dddd, 10.1,9.9, 3.1, 2.4)	55.5 (CH)	3.48 (m, 1H)	54.14 (CH)
8	4.88 (ddd, 11.0, 10.1, 1.8)	71.2 (CH)	4.91 (td, 10.5, 2.0, 1H)	69.62 (CH)
9	9α: 2.83 (dd, 13.4, 11.0) 9β: 2.42 (dd, 13.4, 1.8)	45.0 (CH_2)	9α: 2.84 (m, 1H) 9β: 2.40 (dd, 13.5, 2.0, 1H)	43.72 (CH_2)
10	---	148.1 (q)	---	144.57 (q)
11	---	138.1 (q)	---	137.01 (q)
12	---	170.3 (q)	---	167.81 (q)
13	13: 5.90 (d, 3.1) 13': 5.23 (d, 2.4)	122.3 (CH_2)	13: 5.89 (d, 3.2, 1H) 13': 5.38 (d, 3.2, 1H)	120.54 (CH_2)
14	2.39 (s)	21.5 (CH_3)	2.35 (s, 3H)	20.11 (CH_3)
15	15: 4.85 (dd, 17.7, 1.2) 15': 4.40 (dd, 17.7, 0.8)	63.2 (CH_2)	15: 4.84 (m, 1H) 15': 4.38 (m, 1H)	62.01 (CH_2)
1'	---	172.6 (q)	---	170.62 (q)
2'	3.62 (s)	41.7 (CH_2)	3.62(s, 2H)	40.14 (CH_2)
3'	---	125.8 (q)	---	124.67 (q)
4'/8'	7.12 (d, 8.5)	131.6 (CH)	7.15 (d, 8.4, 2H)	130.54 (CH)
5'/7'	6.74 (d, 8.5)	116.5 (CH)	6.79 (d, 8.5, 2H)	115.40 (CH)
6'	---	158.1 (q)	---	156.70 (q)

Structure elucidation of CiB3b1 (11,13-Dihydrolactupicrin)

CiB3b1 was never fully separated from CiB3b2. The data presented was determined through comparison with data of the purified CiB3b2 (lactupicrin) and close observation of relative integration across the spectrum. The suspected assignment of peaks was then verified utilizing the 2D COSY spectrum, which showed a distinct correlation between a doublet at 1.16 ppm (C13) and a multiplet at 2.60 ppm (C11). The proton at C6 was also shown to couple to the proton at C11 (Figure 14).

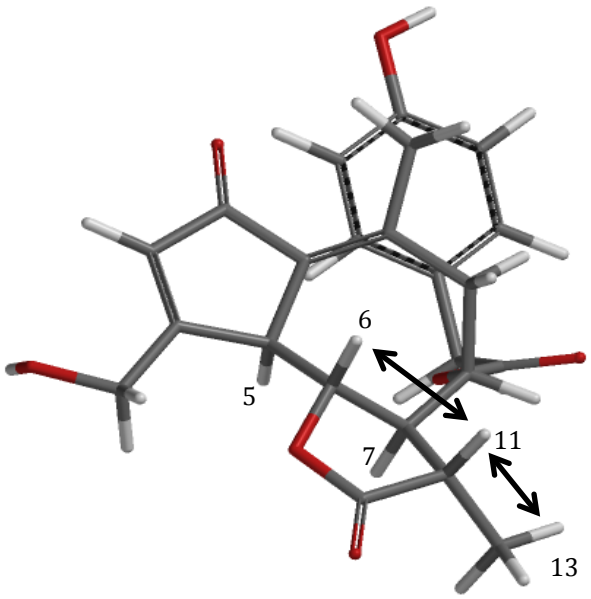


Figure 14: 3D structure of 11,13-dihydrolactucin. Couplings observed in COSY depicted with double-headed arrows.

The values obtained through spectral comparison with CiB3b2 and COSY analysis are compared to the literature values in Table 4.

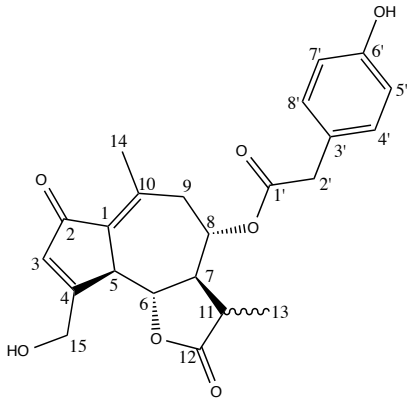


Table 4: ^{1}H and ^{13}C-NMR Data for CiB3b1 compared to literature spectral data				
Position	**Literature [22] CD_3OD**		**Experimental Data* Acetone-D_6**	
	δ ^{1}H-NMR (mult, *J* Hz)	δ ^{13}C-NMR (type)	δ ^{1}H-NMR (mult, *J* Hz)	δ ^{13}C-NMR (type)
1	---	134.3 (q)	---	133.13 (q)
2	---	197.1 (q)	---	194.17 (q)
3	6.40 (s)	133.4 (CH)	6.35 (d, 1.2, 1H)	132.23 (CH)
4	---	179.2 (q)	---	176.44 (q)
5	3.72 (bd, 10.1)	49.9 (CH)	3.73 (d, 10.2, 1H)	48.37 (CH)
6	3.78 (dd, 10.1, 10.1)	82.2 (CH)	3.90-3.85 (m, 1H)	80.47 (CH)
7	2.47 (ddd, 11.8, 10.1, 10.1)	59.4 (CH)	3.48 (m, 1H)	58.07 (CH)
8	4.81 (ddd, 10.8, 10.1, 1.9)	72.4 (CH)	4.83 (td, 10.6, 1.9, 1H)	70.82 (CH_2)
9	9α: 2.80 (dd, 13.6, 10.8) 9β: 2.35 (dd, 13.6, 1.9)	45.4 (CH_2)	2.84 (m, 1H) 2.34 (m, 1H)	43.99 (CH_2)
10	---	148.5 (q)	---	145.12 (q)
11	2.58 (dq, 11.8, 6.7)	41.9 (CH)	2.60 (m, 1H)	40.34 (CH)
12	---	172.8 (q)	---	170.66 (q)
13	1.15 (d, 6.7)	15.4 (CH_3)	1.16 (d, 6.8, 3H)	14.50 (CH_3)
14	2.39 (s)	21.6 (CH_3)	2.35 (s, 3H)	20.15 (CH_3)
15	15: 4.82 (d, 18.0) 15': 4.37 (d, 18.0)	63.2 (CH_2)	4.82 (t, 17.7, 1H) 4.37 (t, 18.5, 1H)	62.00 (CH_2)
1'	---	176.5 (q)	---	174.31 (q)
2'	3.59 (AA')	41.6 (CH_2)	3.60 (s, 2H)	40.06 (CH_2)
3'	---	125.9 (q)	---	124.75 (q)
4'/8'	7.09 (d, 8.4)	131.6 (CH)	7.12 (d, 8.5, 2H)	130.48 (CH)
5'/7'	6.73 (d, 8.4)	116.6 (CH)	6.77 (d, 8.5, 2H)	115.31 (CH)
6'	---	157.9 (q)	---	156.75 (q)

Discussion of Optimized Extraction

The optimized gradient-column extraction method developed during the course of this project greatly reduced the time required to achieve a comprehensive extraction of plant material. The advantages of this method are that the extract is filtered during the course of the extraction process and that the extract is fractionated by solubility as part of the extraction procedure as well. The disadvantages of this method are that it requires the use of a large volume of solvents relative to the amount of plant material and that it would be rather difficult to scale it to accommodate the 60+ g of dried plant material required to obtain reasonable yields of isolated compounds.

In further studies, it would be interesting to observe how the five fractions obtained from the extraction differ in biological activity and if this extraction method allows for more efficient and more comprehensive bioassay-guided fractionation studies. In one day, a reasonable purified product containing primarily 8-deoxylactucin and lactupicrin was obtained from powdered plant material as seen in the HPLC spectrum of CECi4b (Figure 15).

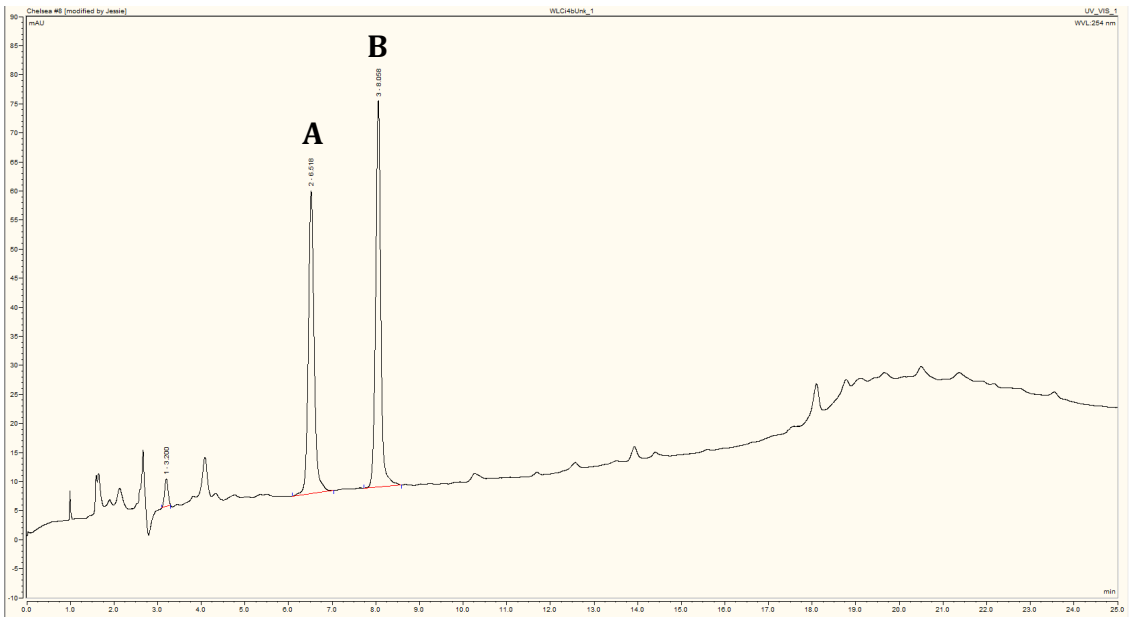
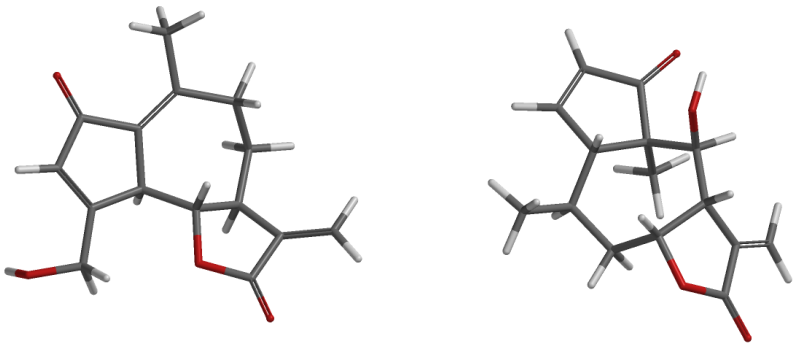


Figure 15: Reverse-phase (C_{18}) HPLC spectrum of CECi4b showing the major constituents as 8-deoxylactucin (Peak A) and lactupicrin (Peak B).

Conclusions

An extraction was conducted on chicory and fractionated following bioassay-guided fractionation to yield two isolated compounds with activity against *T. brucei*. These compounds were identified as 8-deoxylactucin and lactupicrin. A third compound (11,13-dihydrolactupicrin) was also identified but not isolated. 8-Deoxylactucin was identified to be the most potent of the three compounds. 8-Deoxylactucin is structurally similar to helenalin (Figure 16), a sesquiterpene lactone isolated from *Arnica montana*, is also known to exhibit potent activity against *T. brucei*, but it is highly cytotoxic [16].



8-Deoxylactucin Helenalin

Figure 16: The 3D structures of 8-deoxylactucin and helenalin.
A study should be conducted to compare the activities and cytotoxicities of 8-deoxylactucin and helenalin.

Due to time restrictions, the compounds responsible for the anti-viral, anti-inflammatory, and anti-fungal activities were not isolated. More work is needed to pursue these compounds through bioassay-guided fractionation. The fraction CiA7e proved to be a very difficult fraction to fractionate further, but it was found to contain the most potent activity against HSV-1. It would be interesting to see where the HSV-1 activity is found in the fractions obtained from the gradient-column extraction to see if the change in chemical environment of the active compound(s) could aid in the isolation.

REFERENCES

1. MJ Balunas, AD Kinghorn. "Drug Discovery from Medicinal Plants." *Life Sciences*. 2005, 78 (5), 431-441.
2. F Bourgaud. "Production of Plant Secondary Metabolites: A historical perspective." *Plant Science*. 2001, 161 (5), 839-851.
3. S Foster, VE Tyler. "Chicory." *Tyler's Honest Herbal: A Sensible Guide to the Use of Herbs and Related Remedies*. 4th ed. 1999. Pg. 115-117.
4. S Nandagopal, BD Ranjitha Kumari. "Phytochemical and Antibacterial Studies of Chicory (*Cichorium intybus L.*)- A multipurpose Medicinal Plant." *Advan. Bio. Res*. 2007, 1 (1-2), 17-21.
5. I Suntar, EK Akkol, H Keles, E Yesilada, SD Sarker, T Baykal. "Comparative evaluation of traditional prescriptions from *Cichorium intybus L.* for wound healing: Stepwise isolation of an active component by in vivo bioassay and its mode of activity." *J. EthnoPharm.* 2012, 143, 299-309.
6. TA Bischoff, CJ Kelley, Y Karchesy, M Laurantos, P Nguyen-Dinh, AG Arefi. "Antimalarial acitivty of Lactucin and Lactucopicrin: sesquiterpene lactones isolated from *Cichorium intybus L.*" *J. Ethnopharm.* 2004, 95, 455-457.
7. JG Foster, KA Cassida, KE Turner. "In vitro analysis of the anthelmintic activity of forage chicory (*Cichorium intybus L.*) sesquiterpene lactones against a predominantly *Haemonchus contortus* egg population." *Vet. Parasit.* 2011, 180, 298-306.
8. D Mares, C Romagnoli, B Tosi, E Andreotti, G Chillemi, F Poli. "Chicory Extracts from *Cichorium intybus L.* as Potential Antifungals." 2005, 160 (1), 85-91.

9. E Abdel-Sattar, L Maes, MM Salama. “In Vitro Activities of Plant Extracts from Saudi Arabia against Malaria, Leishmaniasis, Sleeping Sickness, and Chaga Disease.” *Phytother. Res.* 2010, 24, 1322-1328.

10. HL Zhang, LH Dai, YH Wu, XP Yu, YY Zhang, RF Guan, T Liu, J Zhao. “Evaluation of Hepatocyteprotective and Anti-hepatitis B Virus Properties of Cichoric Acid from *Cichorium intybus* Leaves in Cell Culture.” *Biol. Pharm. Bull.* 2014, 37 (7), 1214-1220.

11. PN Pushparaj, HK Low, J Manikandan, BKH Tan, CH Tan. “Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats.” *J. Ethnopharm.* 2007, 111, 430-434.

12. JW de Kraker, MCR Franssen, MCF Dalm, A de Groot, HJ Bouwmeester. “Biosynthesis of Germacrene A Carboxylic Acid in Chicory Roots. Demonstration of a Cytochrome P450 (+)-Germacrene A Hydroxylase and $NADP^+$-Dependent Sesquiterpenoid Dehydrogenase(s) Involved in Sesquiterpene Lactone Biosynthesis.” *Plant Physiol.* 2001, 125, 1930-1940.

13. I Merfort. “Review of the analytical techniques for sesqutierpenes and sesquiterpene lactones.” *J. Chromatogr. A.* 2002, 967, 115-130.

14. SS Sue, HM Zhang, XY Liu, et al. “Cloning and characterization of a farnesyl pyrophosphate synthase from *Matricaria recutita L*. and its upregulation by methyl jasmonate.” *Genet. Mol. Res.* 2015, 14(1), 349-361.

15. JP Blakeman, P Atkinson. “Antimicrobial properties and possible role in host-pathogen interactions of parthenolide, a sesquiterpene lactone isolated from

glands of *Chrysanthemum parthenium*." *Physiol. Plant. Path.* 1979, 15(2), 183-190.

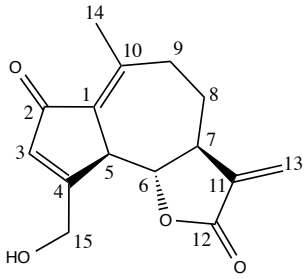
16. G Lyss, TJ Schmidt, I Merfort, HL Pahl. "Helenalin, an anti-inflammatory sesquiterpene lactone from *Arnica*, selectively inhibits transcription factor NF-κB." *Biol. Chem.* 1997, 378 (9), 951-961.
17. A Robinson, TV Kumar, E Sreedhar, VGM Naidu, SR Krishna, KS Babu, PV Srinivas, JM Rao. "A new sesquiterpene lactone from the roots of *Saussurea lappa*: Structure-anticancer activity study." *Bioorg. Med. Chem. Let.* 2008, 18(14), 4015-4017.
18. MZ Abdin, M Israr, RU Rehman, SK Jain. "Artemisinin, a novel antimalarial drug: biochemical and molecular approaches for enhanced production." *Planta Medica*. 2003, 69(4), 289-299.
19. P Rüngeler, V Castro, G Mora, N Gören, W Vichnewski, HL Pahl, I Merfort, TJ Schmidt. "Inhibition of Transcription Factor NF-κB by Sesquiterpen Lactones: a Proposed Molecular Mechanism of Action." *Bioorg. Med. Chem.* 1999, 7, 2343-2352.
20. Stephen Wright. MTSU Biology Department.
21. Jeannie Stubblefield and Alexis Gross. MTSU Biology Department
22. Y Deng, L Scott, D Swanson, JK Snyder, N Sari, H Dogan. "Guaianolide Sesquiterpene Lactones from *Cichorium intybus* (Asteraceae)." *Z. naturforsch.* 56b, 787-796 (2001).
23. Erin Park. MTSU Biology Department.

24. ND Yuliana. M Jahangir, R Verporte, YH Choi. “Metabolomics for the rapid dereplication of bioactive compounds from natural sources.” *Phytochem. Rev.* 2013. 12. 293-304.

APPENDICES

Appendix A

Spectroscopy Data for CiB3a (8-Deoxylactucin)



-NMR

-^{1}H-NMR (Acetone-d_6)

-^{13}C-NMR (Acetone-d_6)

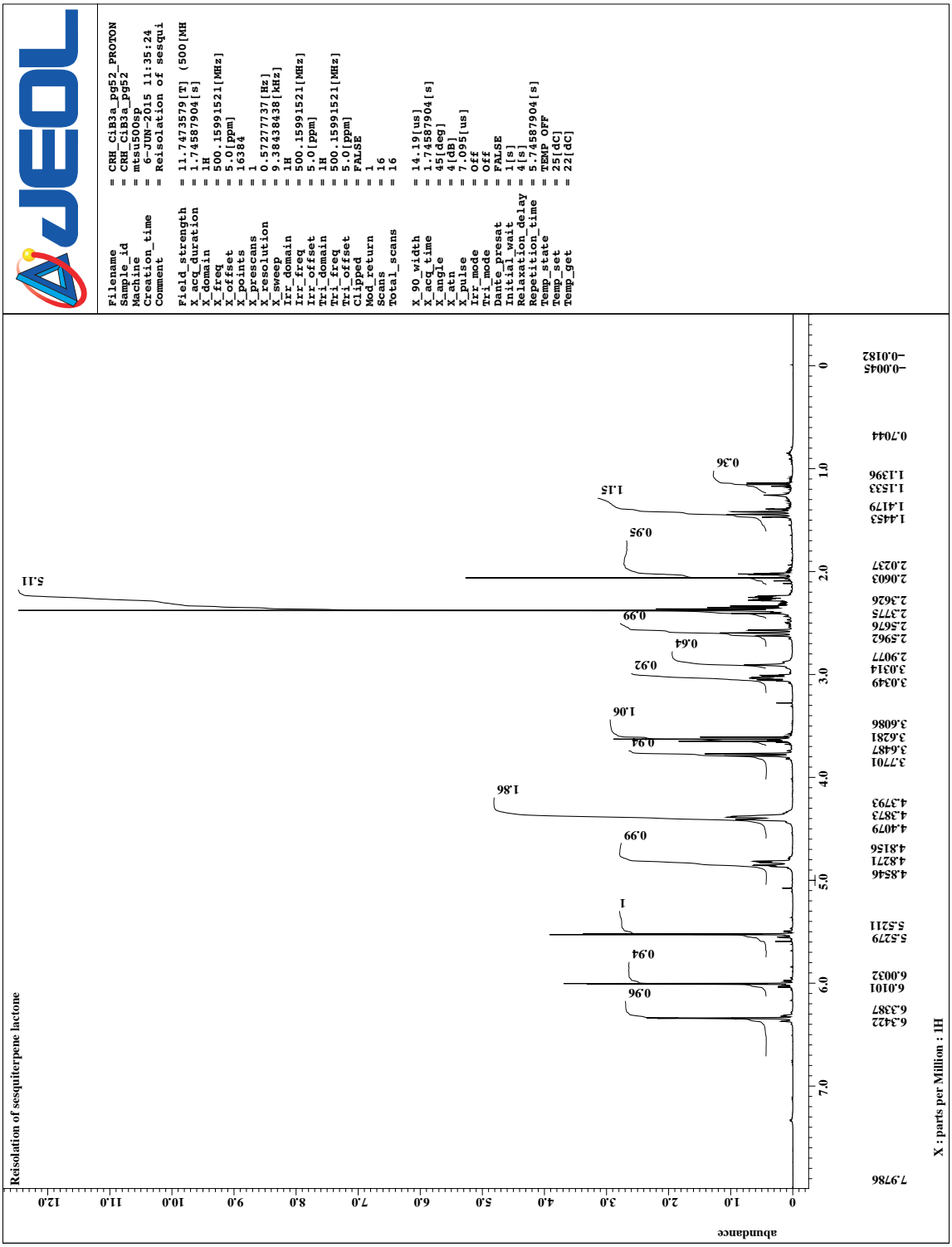
-DEPT$_{135}$ (Acetone-d_6)

-COSY (Acetone-d_6)

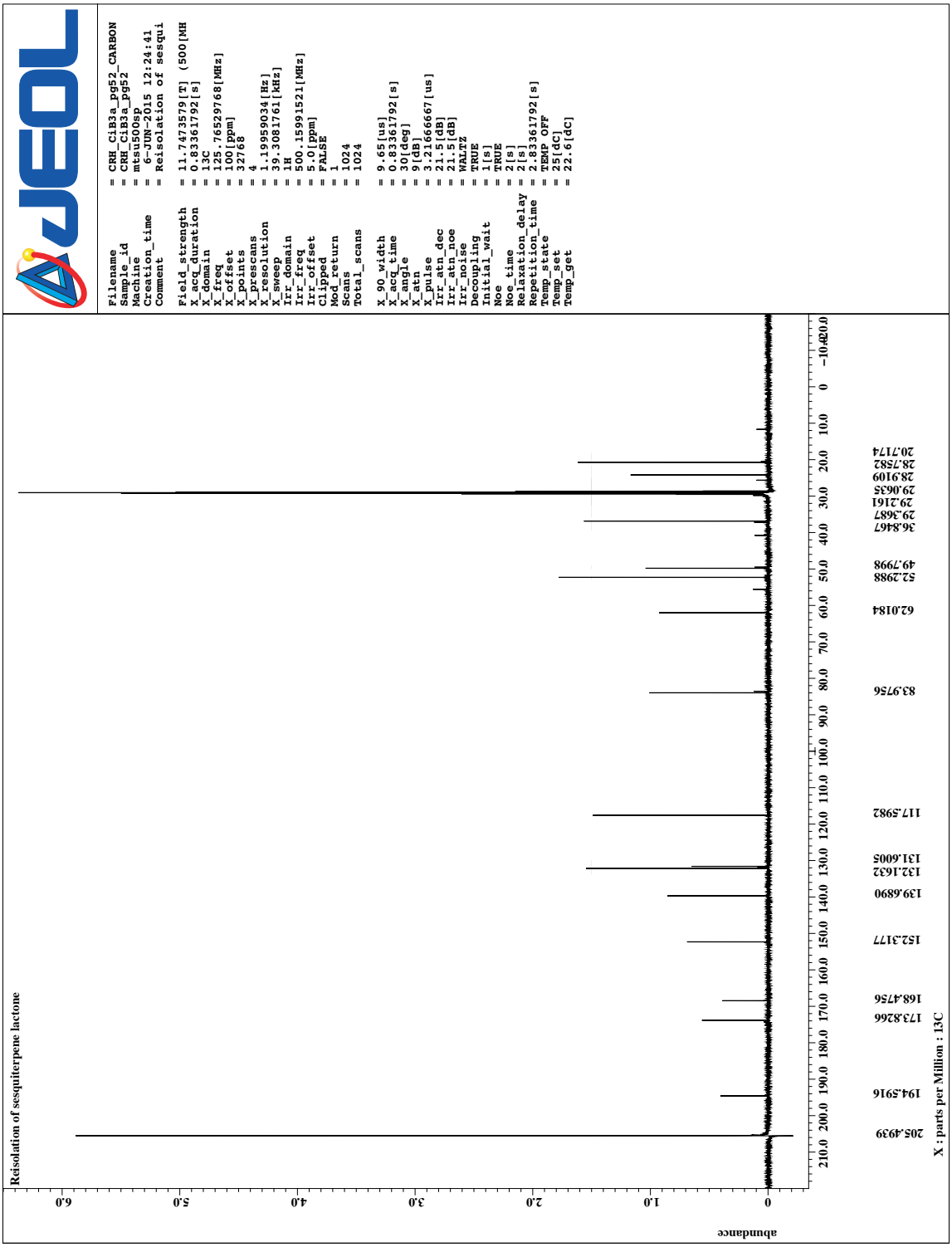
-HMQC (Acetone-d_6)

-HMBC (Acetone-d_6)

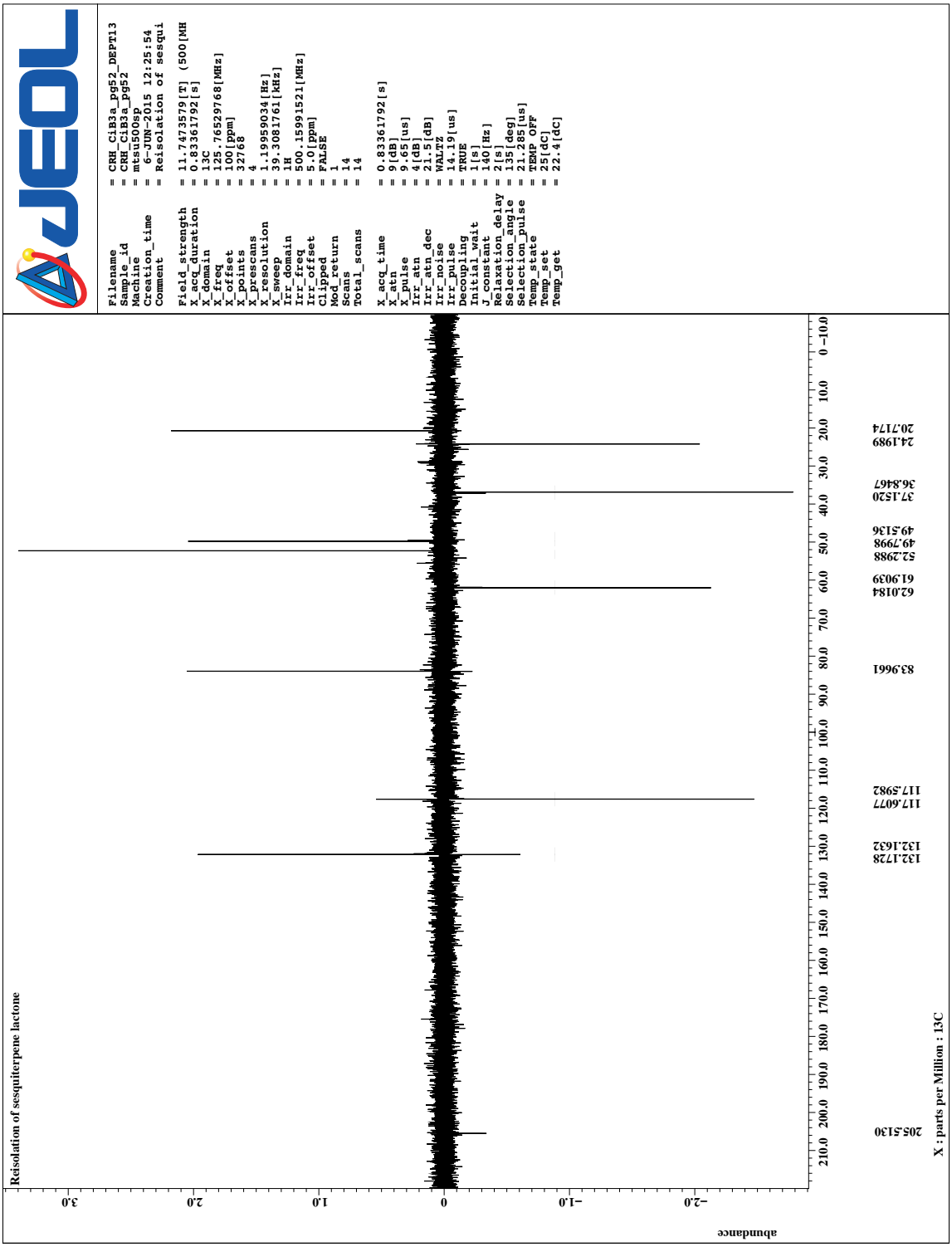
^{1}H-NMR (Acetone-d_6)



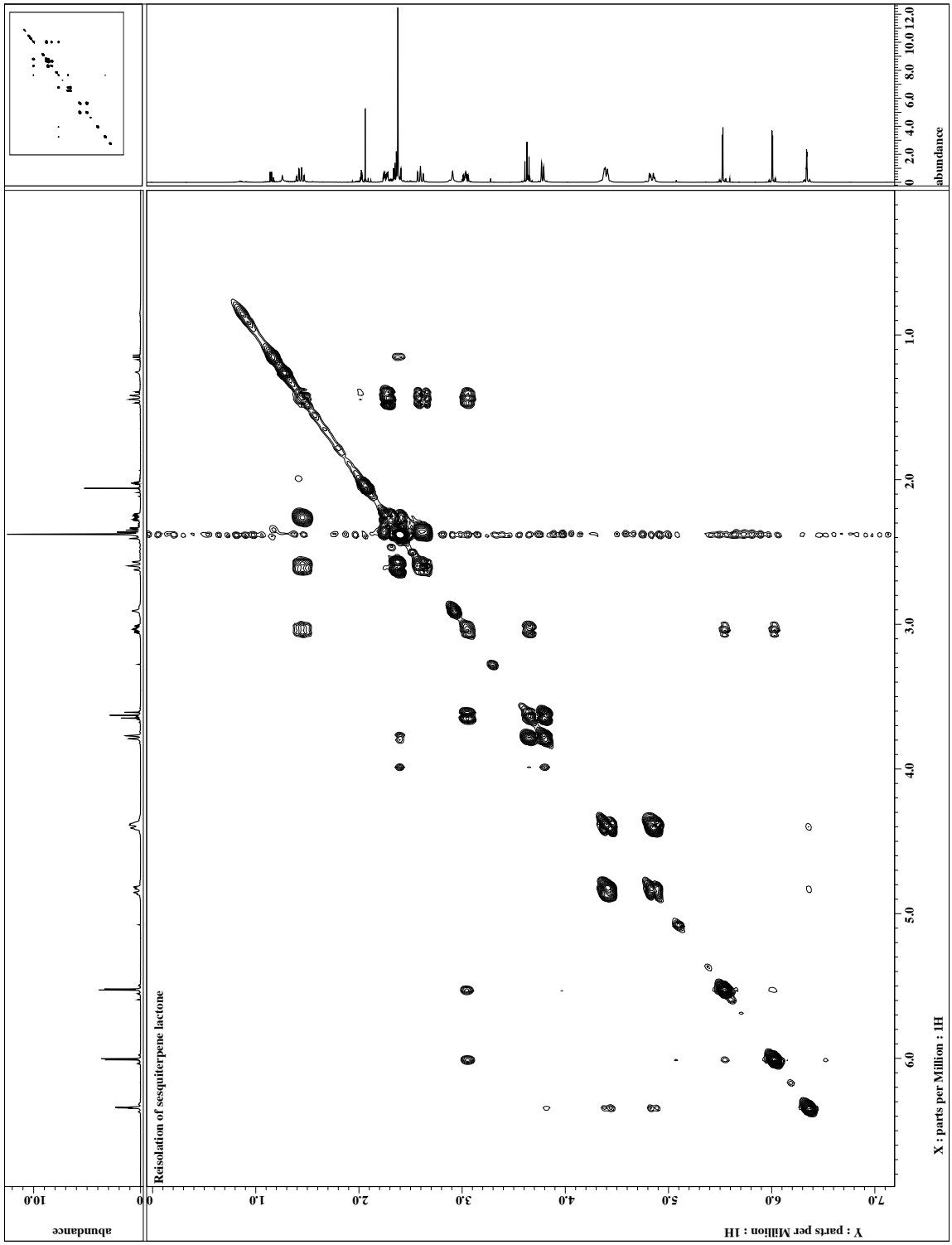
^{13}C-NMR (Acetone-d_6)



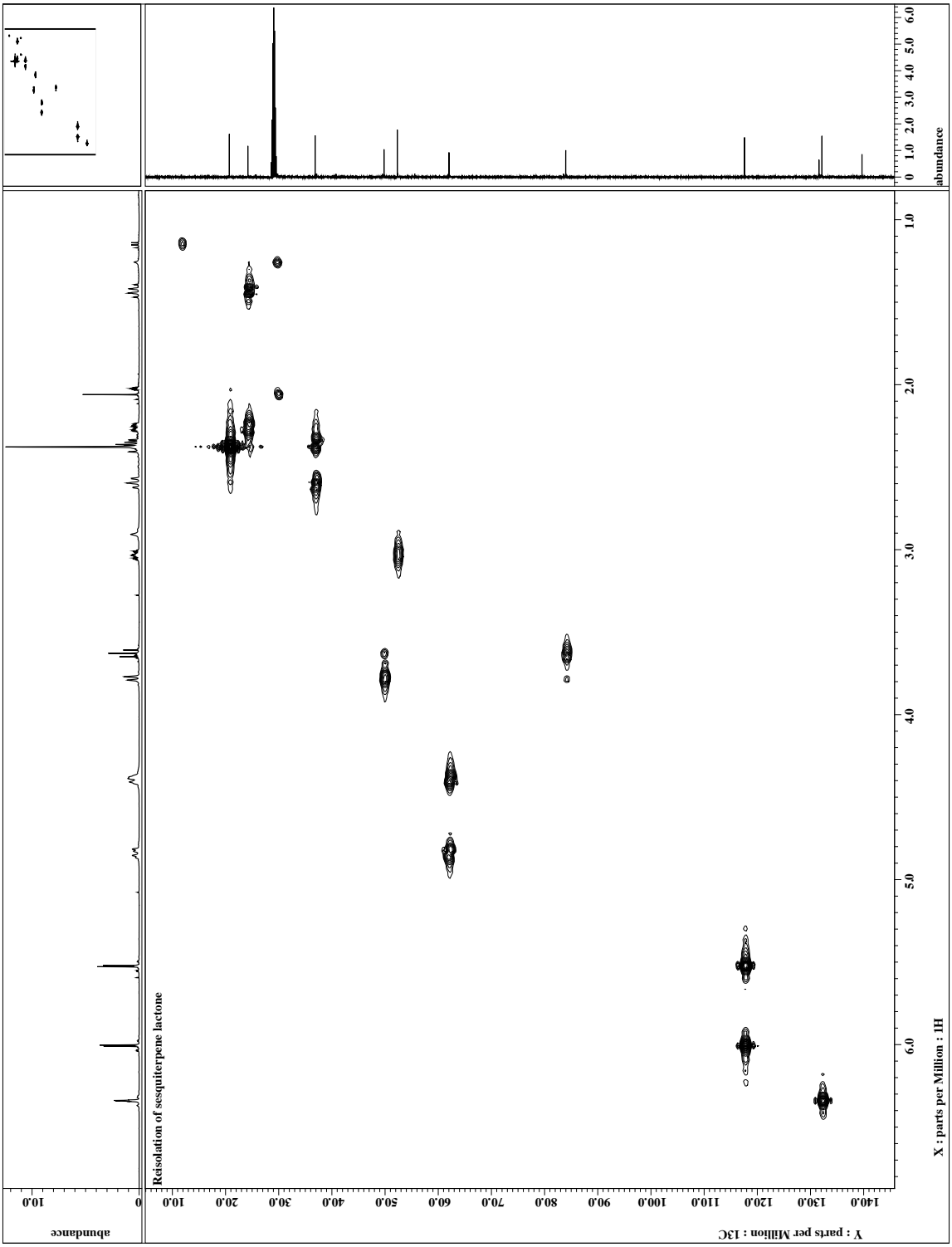
DEPT$_{135}$ (Acetone-d$_6$)



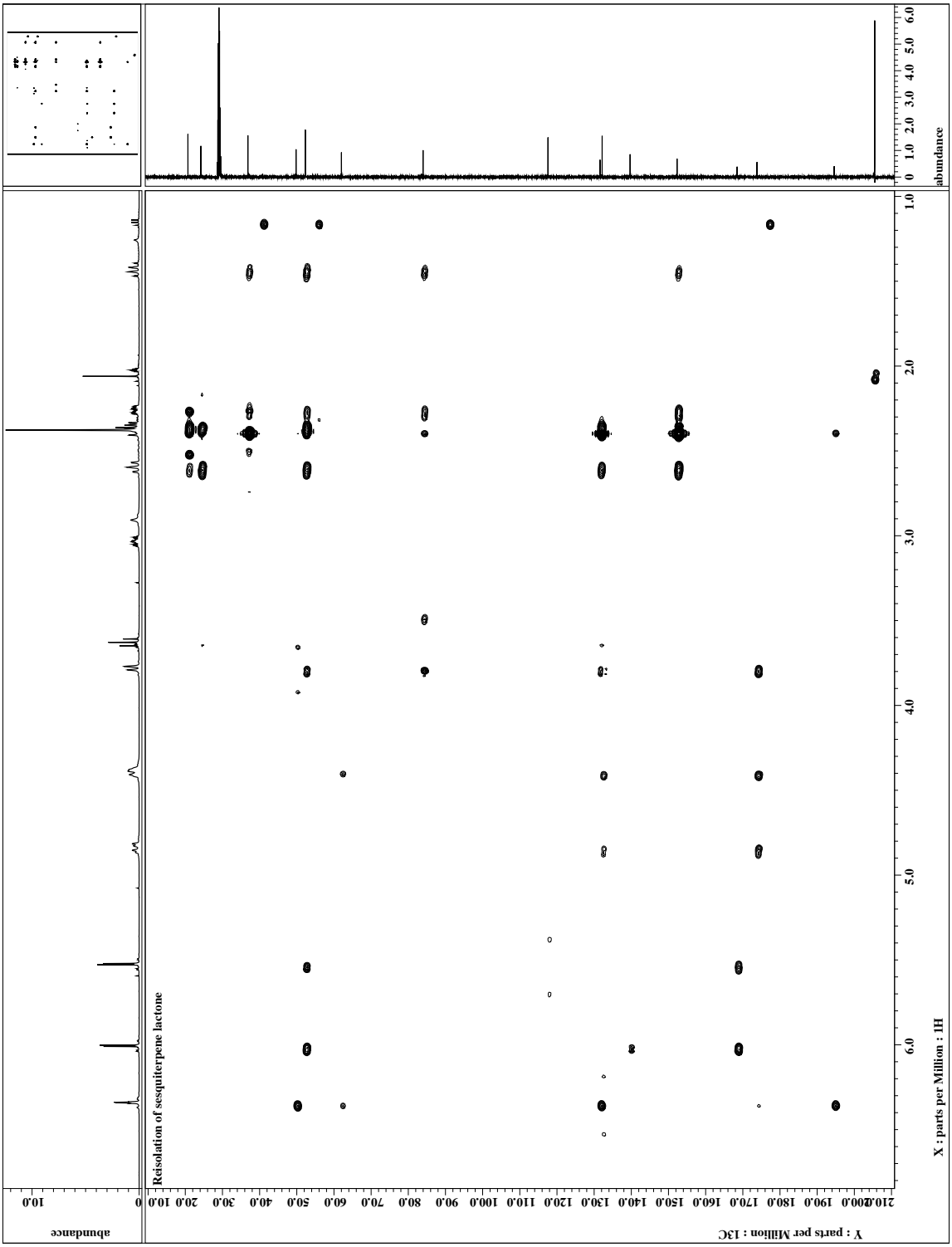
COSY (Acetone-d_6)



HMQC (Acetone-d_6)

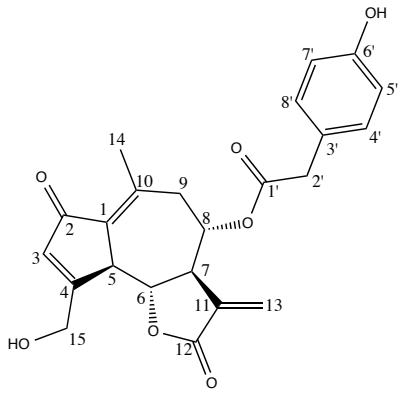


HMBC (Acetone-d_6)



Appendix B

Spectroscopy Data for CiB3b2 (Lactupicrin)



-NMR

-^{1}H-NMR (Acetone-d_6)

-^{13}C-NMR (Acetone-d_6)

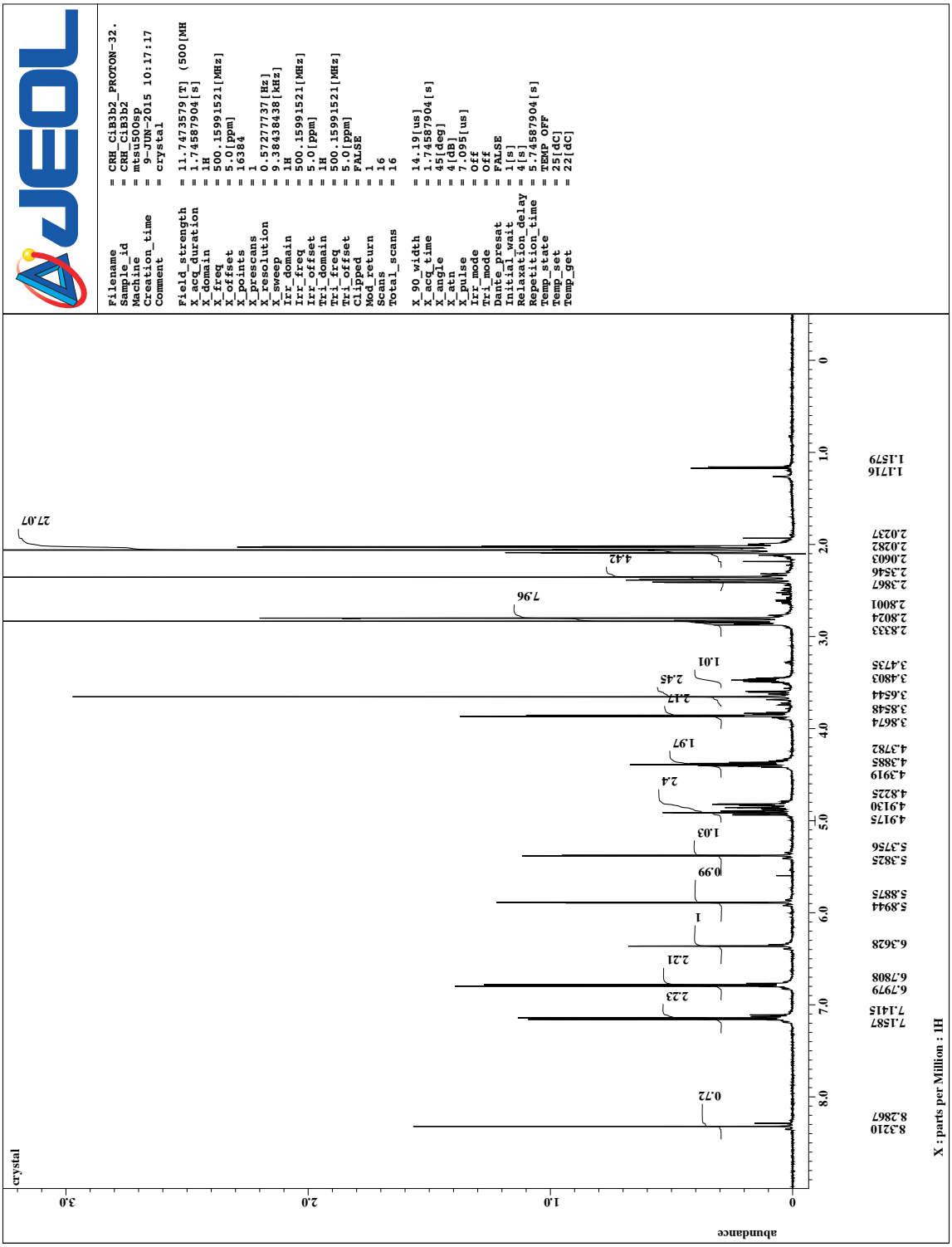
-DEPT$_{135}$ (Acetone-d_6)

-COSY (Acetone-d_6)

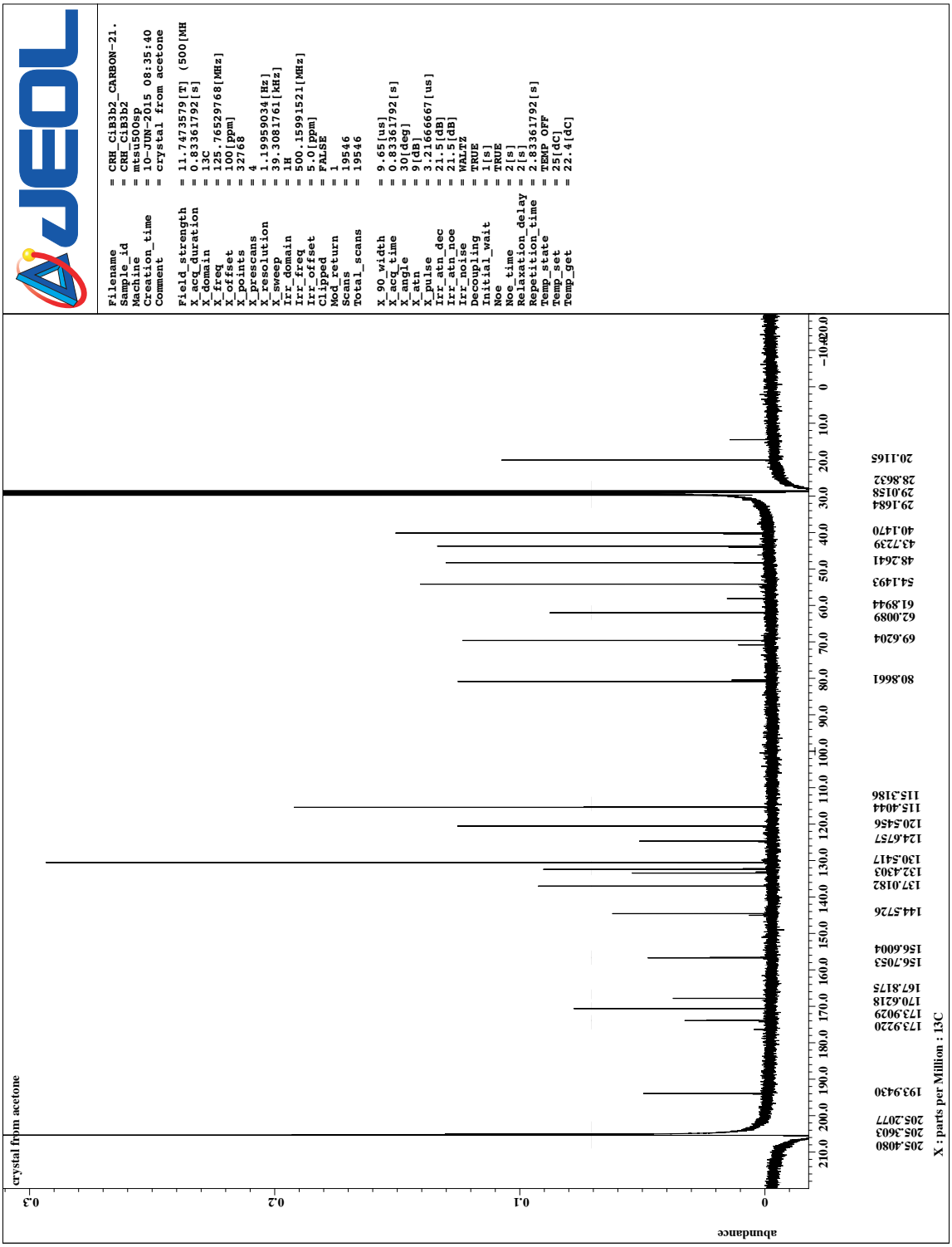
-HMQC (Acetone-d_6)

-HMBC (Acetone-d_6)

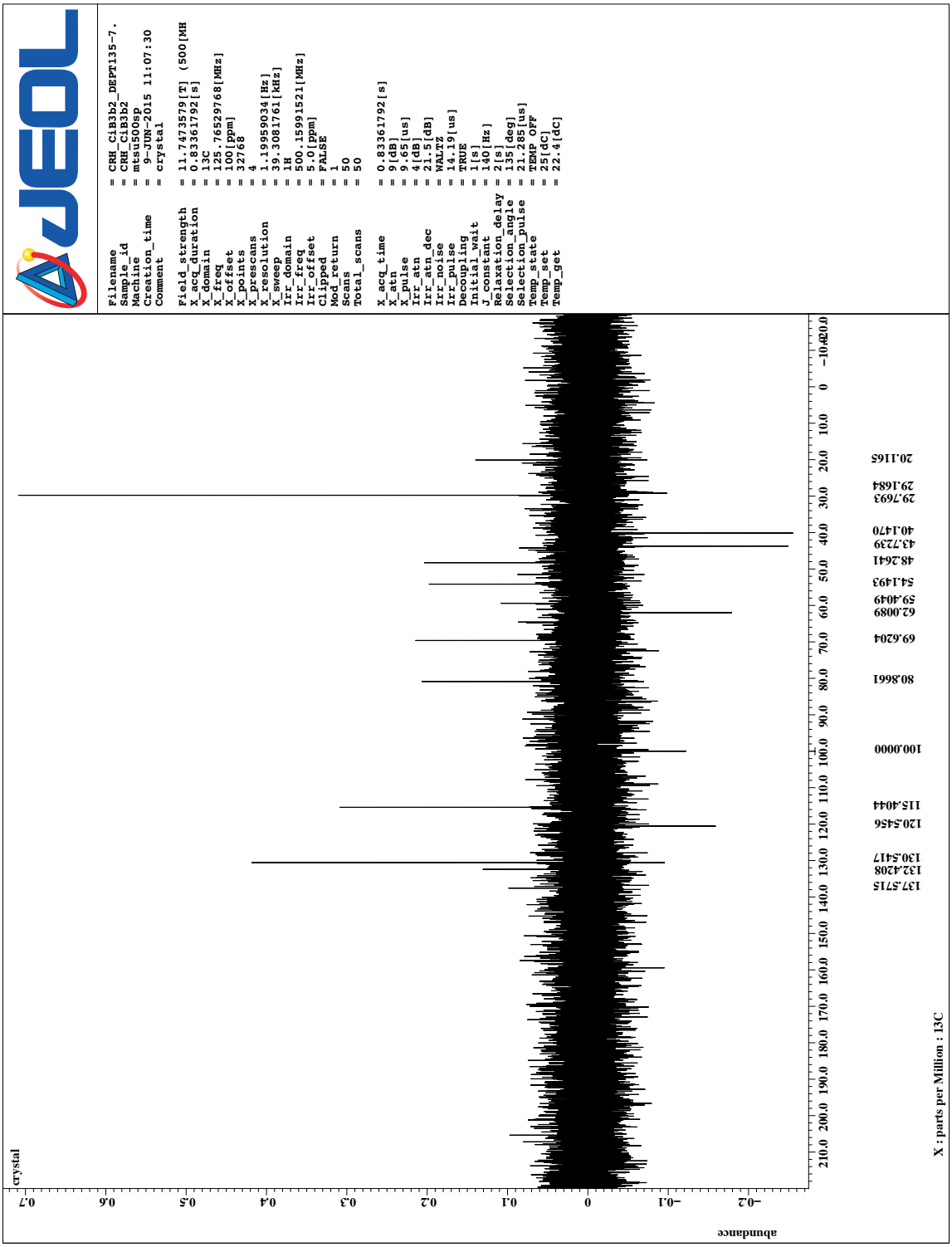
^{1}H-NMR (Acetone-d_6)



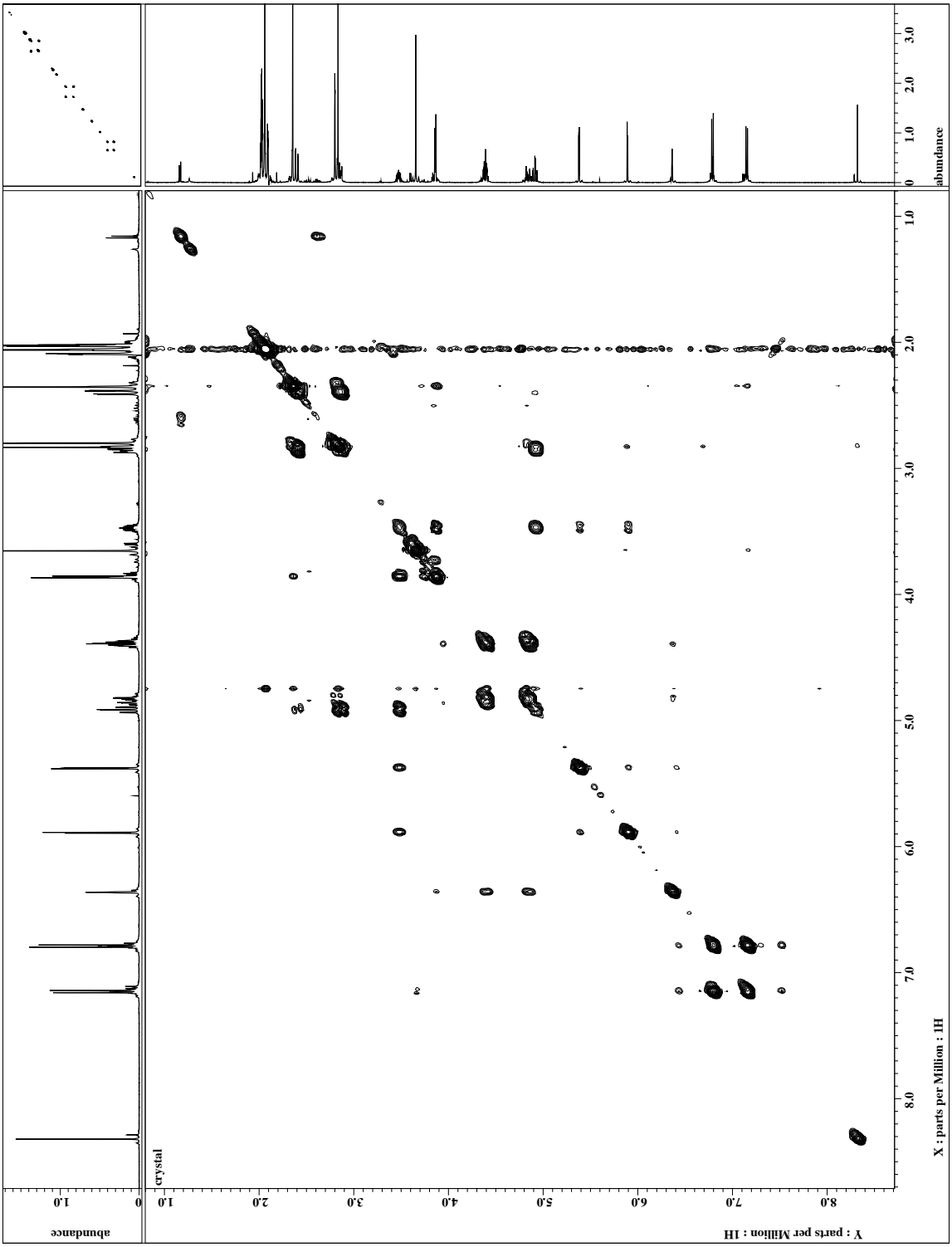
^{13}C-NMR (Acetone-d_6)



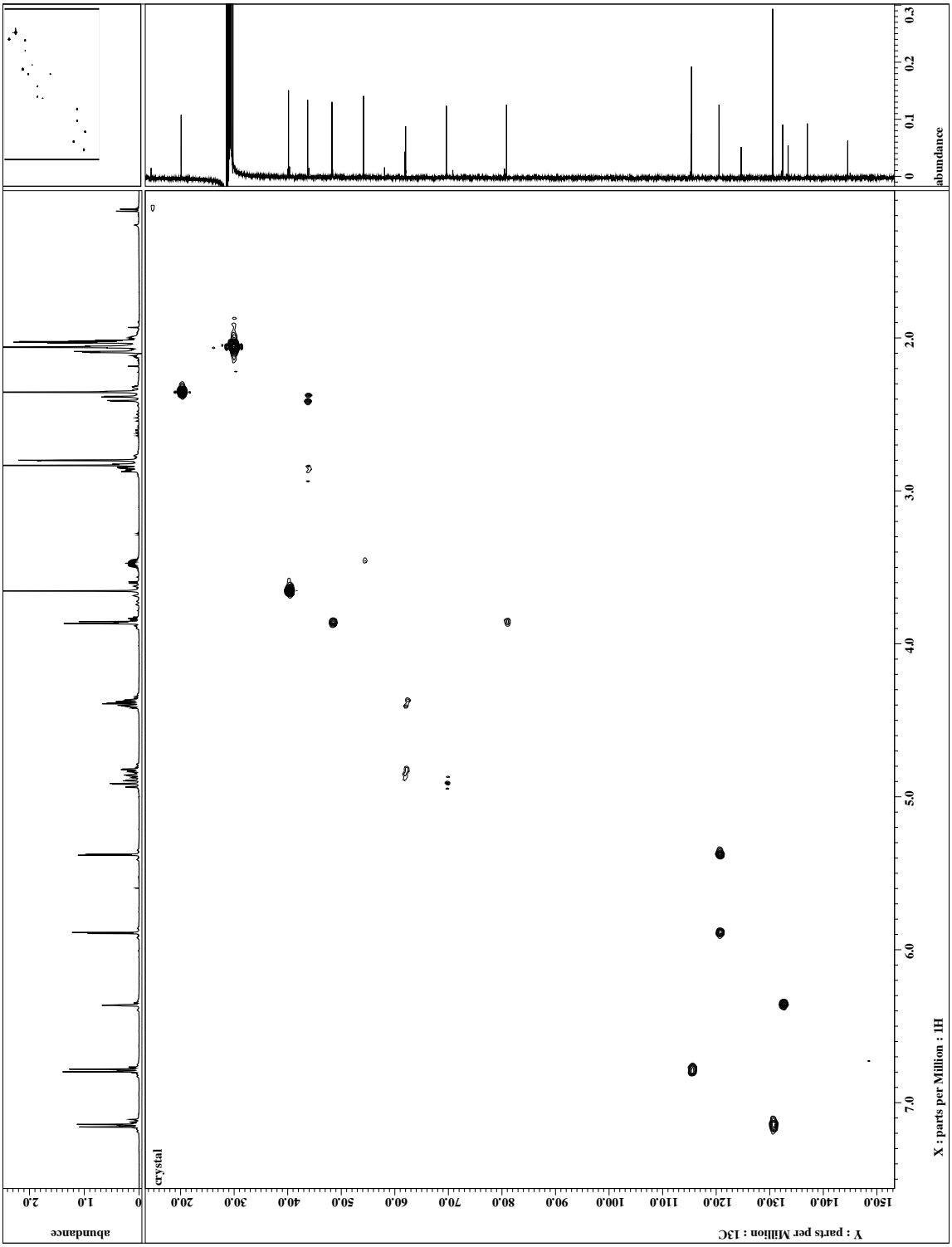
DEPT$_{135}$ (Acetone-d$_6$)



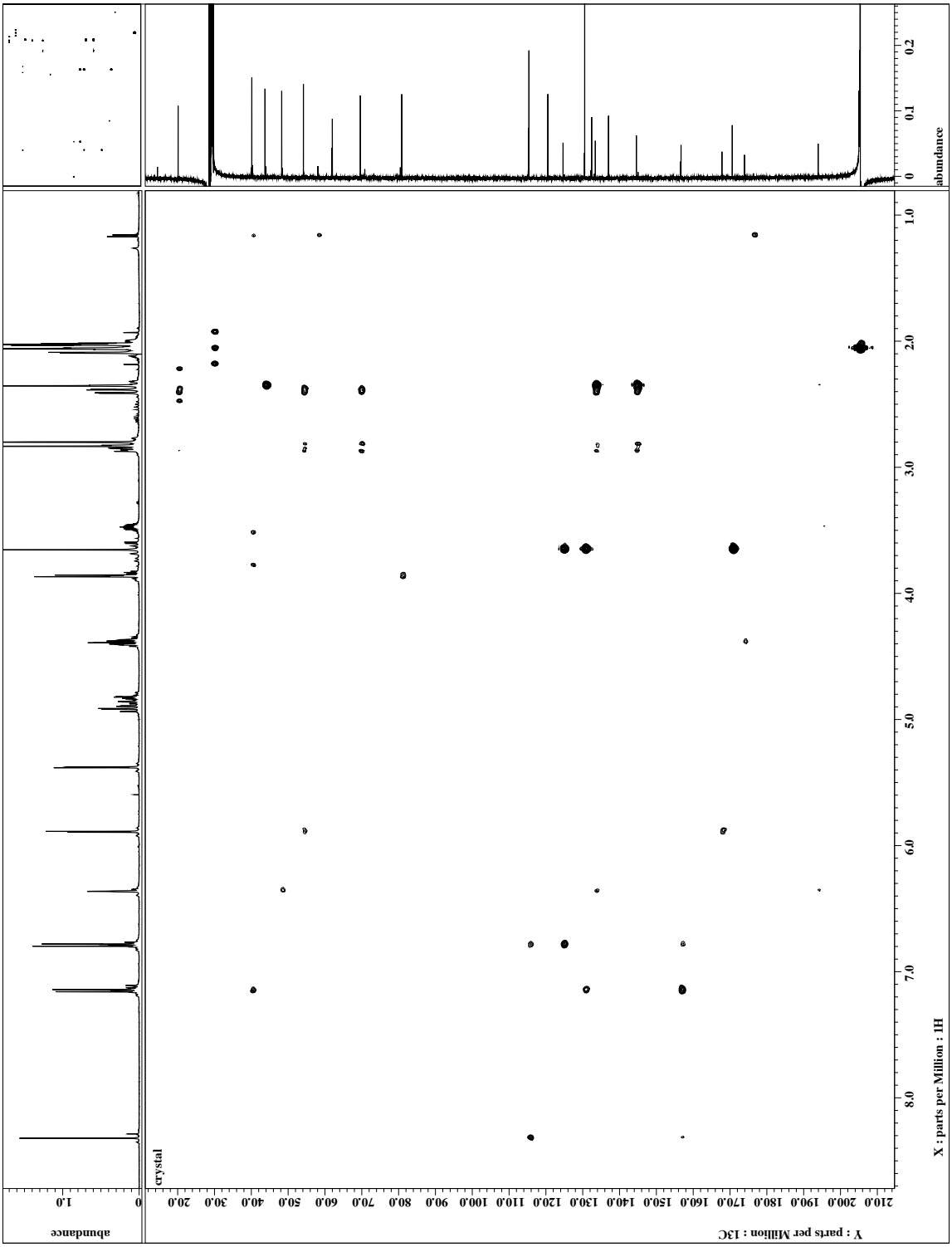
COSY (Acetone-d_6)



HMQC (Acetone-d_6)

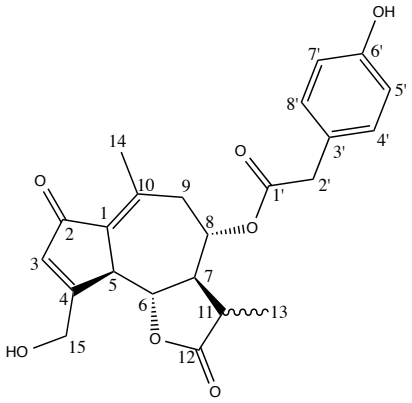


HMBC (Acetone-d_6)



Appendix C

Spectroscopy Data for CiB3b1 (11,13-Dihydrolactupicrin)



-NMR

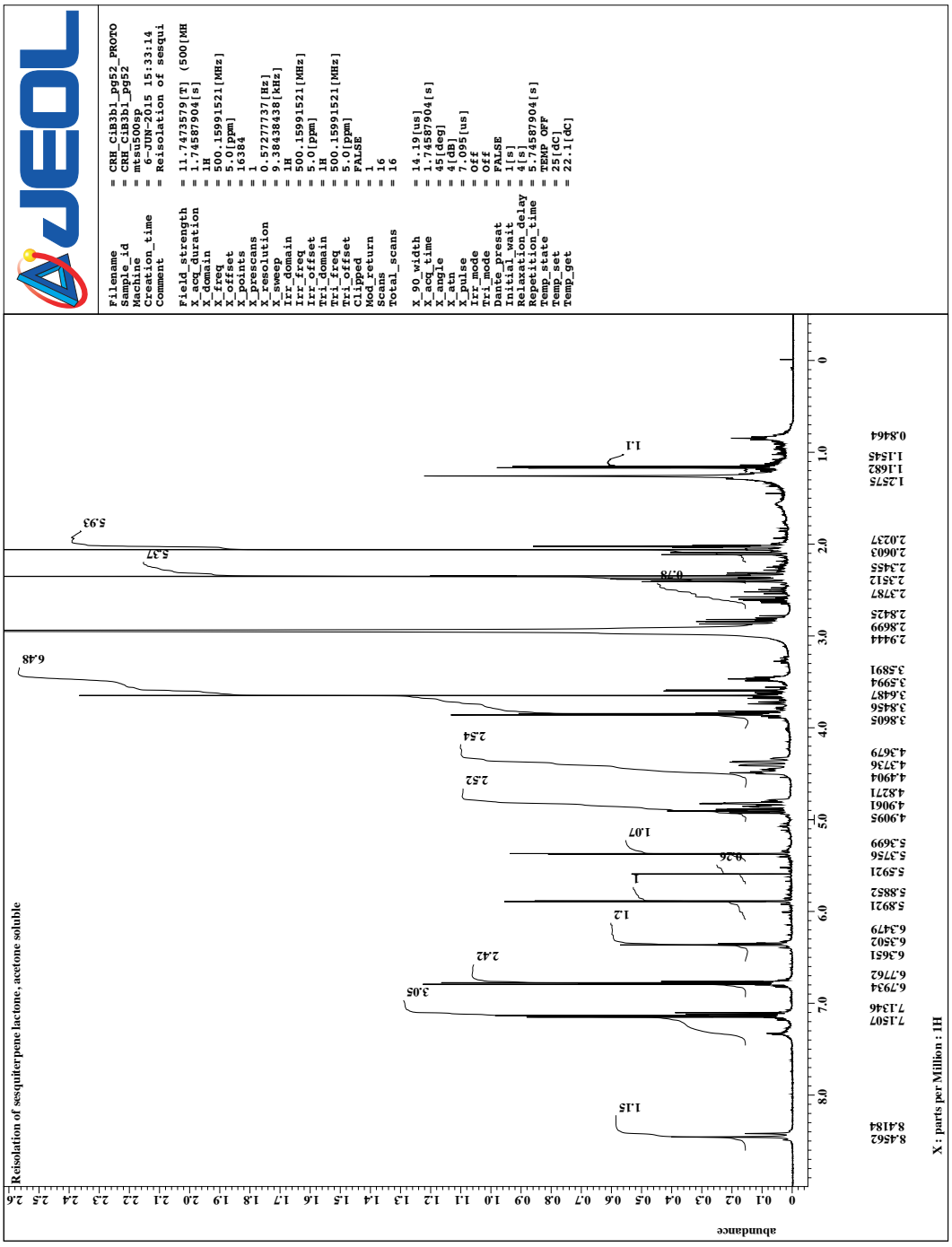
-^{1}H-NMR (Acetone-d_6)

-^{13}C-NMR (Acetone-d_6)

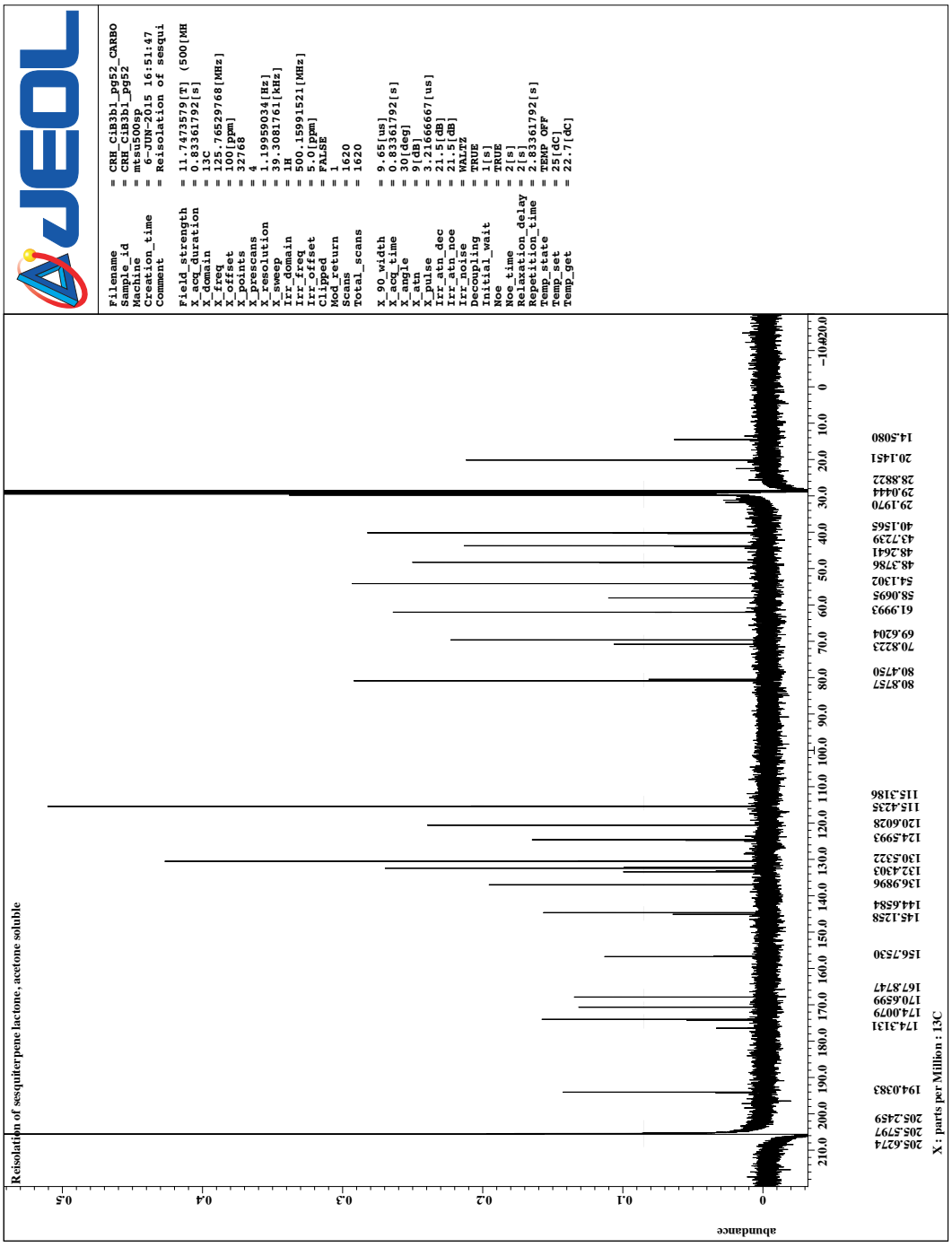
-$DEPT_{135}$ (Acetone-d_6)

-COSY (Acetone-d_6)

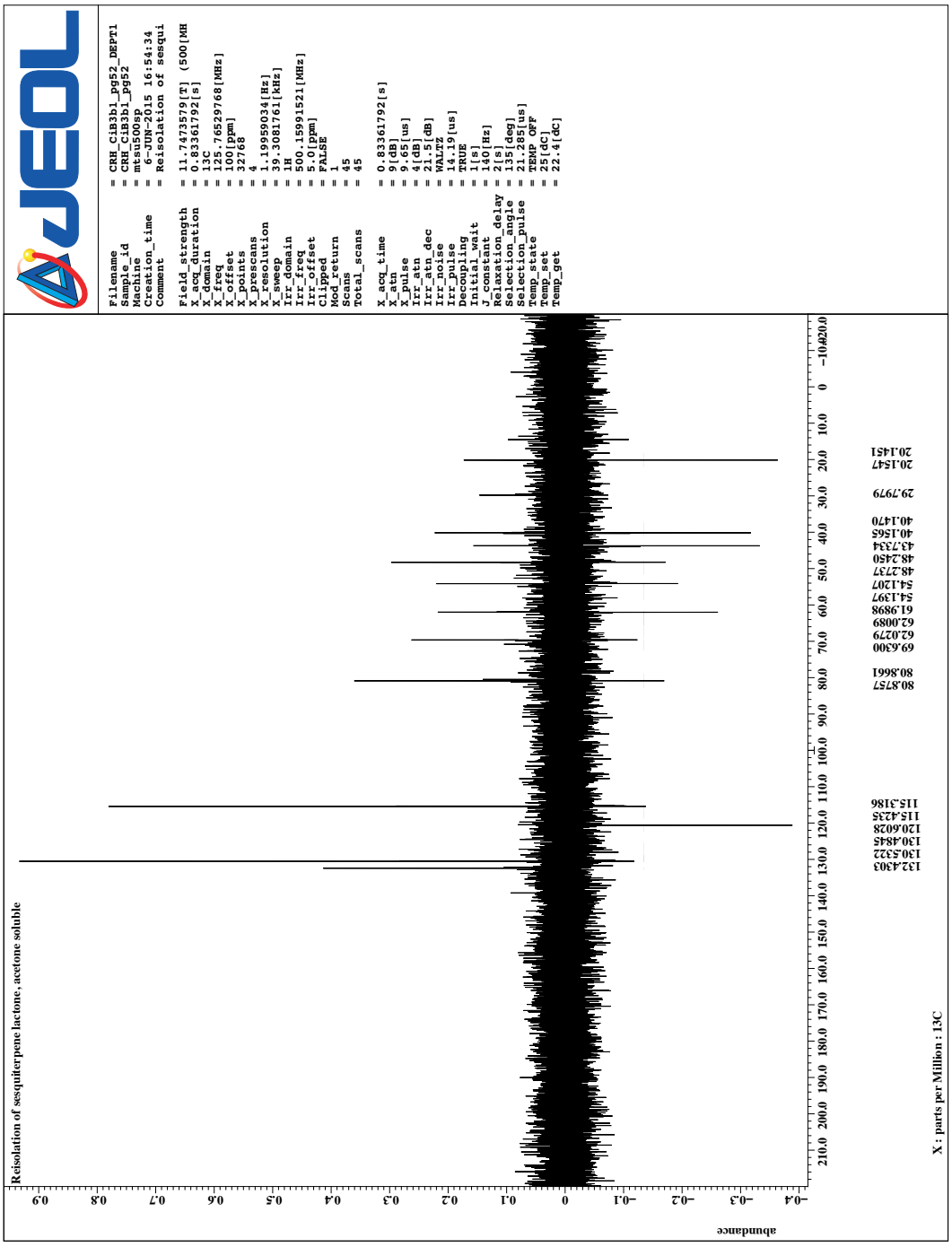
^{1}H-NMR (Acetone-d_6)



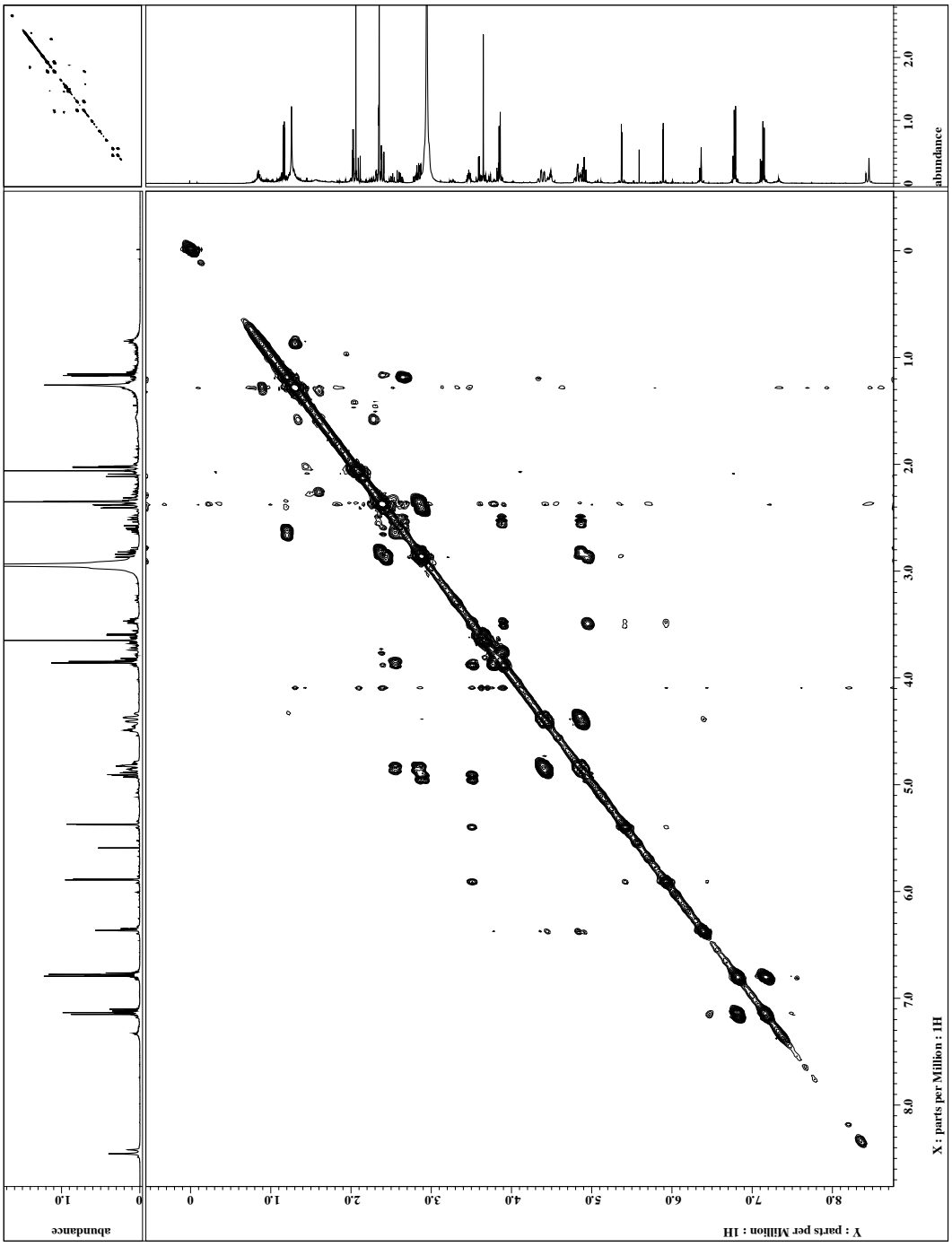
^{13}C-NMR (Acetone-d_6)



DEPT$_{135}$ (Acetone-d$_6$)



COSY (Acetone-d_6)



Appendix D

Trypanosoma Brucei Assay Data

Assay Results Against *Trypanosoma brucei*

Sample	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.562 µg/ml	0.390 µg/ml	0.195 µg/ml	0.097 µg/ml	0.048 µg/ml	0.024 µg/ml	0.012 µg/ml	0.0065 µg/ml	0.003 µg/ml	IC50
CiB3a	84.70	89.48	91.31	92.54	92.97	92.53	93.21	92.53	17.51	-4.04	-3.63	-1.48	0.38	-0.03	0.13
CiB3a	84.62	89.99	91.86	93.09	93.57	93.35	93.36	42.43	0.86	-1.84	-1.29	0.30	0.09	1.32	0.21
CiB3a	83.95	88.86	91.14	92.16	92.70	93.37	92.76	92.92	14.34	-2.85	-1.25	-0.12	1.52	1.66	0.13
CiB3a	85.15	89.45	91.19	91.94	92.38	65.77	-1.27	-0.48	-0.22	-2.35	-1.47	-1.47	-0.77	-0.52	1.46
CiB3a	84.57	89.05	90.71	91.54	91.92	91.22	-2.69	-0.80	-0.28	-2.09	-2.58	-1.37	-1.38	0.12	0.74
CiB3b1	86.01	89.90	91.63	91.66	20.18	-3.54	-0.91	0.54	1.02	-2.93	-2.29	-1.77	0.52	-0.48	4.03
CiB3b1	86.89	91.12	86.81	31.96	2.27	-2.01	-1.31	0.89	1.17	-2.21	-1.03	-0.71	0.70	0.65	7.69
CiB3b1	86.73	90.12	91.64	83.15	27.08	-2.83	-0.18	1.35	2.33	-4.48	-2.46	-1.60	0.50	1.42	4.13
CiB3b1	85.58	4.03	-1.35	-0.95	-1.06	-1.86	-0.39	0.51	0.32	-1.09	-0.48	-0.04	0.44	0.87	38.98
CiB3b1	86.61	46.02	-3.82	-1.54	0.22	-1.98	-0.76	-0.65	1.00	-2.27	-2.10	-0.56	-0.22	1.57	26.91
CiB3b2	82.95	88.34	90.94	91.85	60.28	-2.50	-0.77	-0.29	1.31	-2.97	-3.81	-1.80	0.32	0.77	2.95
CiB3b2	82.22	86.83	13.58	-1.03	1.53	-3.14	-0.62	0.51	2.00	-1.64	-0.61	0.31	0.86	1.88	17.72
CiB3b2	79.59	86.73	90.13	91.20	15.88	-2.71	1.62	3.09	3.58	-2.95	-0.66	0.38	1.86	1.33	4.18
CiB3b2	78.20	37.92	-3.12	-1.28	-1.00	-4.63	-3.06	-2.03	-2.37	-3.89	-3.85	-2.86	-2.99	-1.70	31.02
CiB3b2	78.32	86.08	71.53	-2.55	0.07	-2.36	-1.37	-0.90	-0.27	-3.00	-2.63	-1.08	-0.95	0.96	11.85