ANTIDIABETIC COMPOUNDS FROM *HYPOXIS COLCHICIFOLIA* AND *TERMINALIA SERICEA*

Thesis submitted in fulfilment of the requirements for the degree

Master of Science

By

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ABSTRACT

In this project two medicinal plant species, namely *Hypoxis colchicifolia* Bak (Hypoxidaceae) and *Terminalia sericea* Burch. (Combretaceae), have been investigated and different compounds isolated and characterised. The aim of this study was to investigate the phytochemistry and antidiabetic activity of *H. colchicifolia* and *T. sericea*.

H. colchicifolia (*H. latifolia*) is after *H. hemerocallidea* (African Potato) the second most important *Hypoxis* medicinal species with commercial value in South Africa. From the methanol extract of the corms, four phenolic derivatives, hypoxoside, a mixture of dehydroxyhypoxoside A and B and bis-dehydroxyhypoxoside were isolated, as well as an environmental impurity bisphenol A diglycidyl ether. This is the first study on the isolation of the dehydroxyhypoxoside B. The methanol extract of *H. colchicifolia* showed glucose lowering effects and low toxicity *in vitro* against C2C12 muscle cells and Chang liver cells. Of the pure compounds, only the mixture of dehydroxyhypoxoside A and B showed an increase in glucose utilization by the muscle cell line C2C12.

T. sericea is an important plant in traditional medicine and is in the top 50 most important African medicinal plants. From the methanol extract of the roots, one known compound, sericic acid, was isolated. The methanol extract of *T. sericea* roots showed an increase in glucose utilization by C2C12 muscle cells and Chang liver cells but the extract also showed some cytotoxicity towards these cells.

Structural elucidation of the different compounds was achieved by using NMR spectroscopy in one and two dimensions, high-resolution mass spectrometry, infrared spectroscopy and ultraviolet spectroscopy.

PREFACE

The experimental work described in this dissertation was carried out in the School of Chemistry, Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Fanie van Heerden.

I hereby declare that these studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

Signed ……

Jaime T. Cumbe

Hereby certify that this statement is correct.

Signed …….

Professor Fanie R. van Heerden (**Supervisor**)

ACKNOWLEDGEMENTS

I express my thanks to my Supervisor Professor F.R. van Heerden for her guidance throughout this study. I would to thank to all my colleagues in Organic Chemistry and the staff for their support. A special thanks to Mr Craig Grimmer for his continuous support and most efficient running of the NMR spectrometers.

I am very grateful to Professor Siegfried E. Drewes, for helping me in the collection and identification of plants. I would also like to thank Prof. Musabayane and his research group for collaboration in this project in testing the biological activity of the samples.

My parents for the abundant love and for always have been in my side, my family and friends for their moral support.

A very special thanks to my fiancée Mrs Halima that even the distance never let me feel so, I love you. I want to dedicate this title to my sister who passed without I say last god bye, rest in peace sister.

My employer, Eduardo Mondlane and SIDA-SAREC of Sweden for the financial support.

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ABBREVIATIONS

ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AD	Years following after Christ in the Julian and Gregorian calendars
AMU	Atomic mass units
BC	Before Christ
BuOH	n-Butanol
Calcd.	Calculated
ºC	Degrees Celsius
^{13}C	Carbon-13
C2C12	C2C12 Mouse myoblast cell line
cm^{-1}	Wavenumber
COSY	Correlation spectroscopy
DCM	Dichloromethane
DEPT	Distortionless enhancement by polarization transfer
DMEM	Dulbecco's modified Eagle's medium
DPPH	1,1-Diphenyl-2-picrylhydrazyl
EMEM	Eagle's minimal essential medium
EtOAc	Ethyl acetate
EtOH	Ethanol
FDA	Food and Drug Administration (United States of America)

IR	Infrared spectroscopy
g	Grams
GDIS	Glucose-dependent insulin secretion
Glu	Glucose
GPLP-1	Glucagon-like peptide-1 agonist
h	Hour(s)
^{1}H	Proton
HCT 116	HCT 116 Human colon carcinoma cell line
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple-bond correlation
HRMS	High-resolution mass spectrometry
HPLC	High-performance liquid chromatography
HSQC	Heteronuclear single-quantum correlation
Hz	Hertz
IC_{50}	Half maximal inhibitory concentration
K-ATP	Adenosine triphosphate-sensitive potassium channel
LR-MS	Low-resolution mass spectrometry
MCF-7	MCF-7 Breast cancer cell line
MeOH	Methanol
mg mL^{-1}	Microgram per milliliter

MIC	Minimum inhibitory concentration
MS	Mass spectrometry
PC 3	Human prostate cancer cell lines
PDA	Photodiode-array detector
PPARγ	Peroxisome proliferator-activated receptor gamma
R_t	Retention time
USA	United States of America
STZ	Streptozotocin
UV	Ultraviolet–visible
μL	Microlitre

CHAPTER 1: INTRODUCTION

1.1 PLANTS AS A SOURCE OF MEDICINAL COMPOUNDS

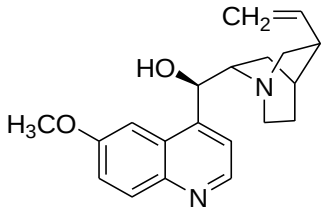
Natural products were the basis of ancient systems of traditional medicine, such as the Chinese, Ayurvedic and Egyptian systems (3000 BC) (Doughari and Rao, 2012; Sarker et al., 2006). Sarker et al. (2006) give (in their book *Natural Products Isolation*) a brief history on the evolution of medicinal natural products:

- Ebers Papyrus (1550 BC) listed a series of crude drugs from natural sources (e.g. castor seeds and gum arabic).
- Hippocrates (450 - 377 BC), Theophrastus (370 - 287 BC) and Pliny (23 - 79 AD) described several plants and animals that could be used in medicine.
- Dioscorides (60-80 AD) presented in his book De Materia Medica a description of over 600 medicinal plants.
- Galen (131-200 AD) practiced botanical medicines (Galenicals) and made it popular in Europe.
- Kraüterbuch (15th century) presented information and pictures of medicinal plants.

According to the World Health Organization (WHO, 2003) about 80% of people in developing countries rely on traditional plant-based medicines for their primary health care. In 1998 it was estimated that the commercial value of ethnomedicinal plants traded in the province of Kwazulu-Natal in South Africa was R60 million. In addition, most households, especially in the rural areas, spend between 4 and 8% of their annual income on traditional medicinal services (Mulholland and Drewes, 2004).

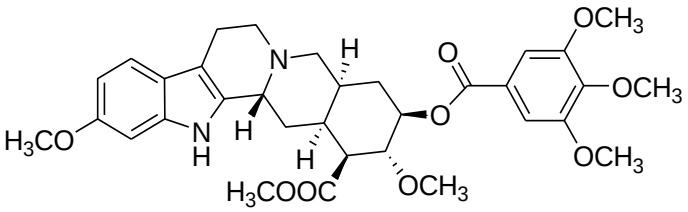
In most plants, the active compounds are secondary metabolites. Secondary metabolites are defined as small molecules (molecular weight <2000 amu) produced by an organism that are not strictly necessary for the survival of the organism. These compounds are responsible for protecting plants against microbial infections or infestations by pests (Doughari and Rao, 2012; Sarker et al., 2006). These secondary metabolites are often referred to as natural products. Natural products isolated from plants and microorganisms have played a vital role as a source of lead compounds for the pharmaceutical industry.

The isolation of active compounds from plants started early in the nineteenth century when compounds such quinine (**1.1**) were obtained as pure compounds. Quinine (**1.1**) is an antimalarial compound that was isolated from the bark of the *Cinchona* tree by the French scientists Cavetto and Pelletier (Henry, 1949).



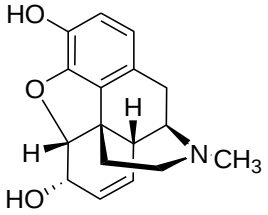
1.1 Quinine

The Indian subcontinent is a good example of an extensive indigenous pharmacopoeia, including the Ayurvedic, Unani, and folkloric medical systems, which has already supplied drugs such as reserpine (**1.2**) (antihypertensive and tranquilizer), from *Rauvolfia serpentina*. Reserpine is also reported to be hypoglycaemic in normal animals (Marles and Farnsworth, 1995).

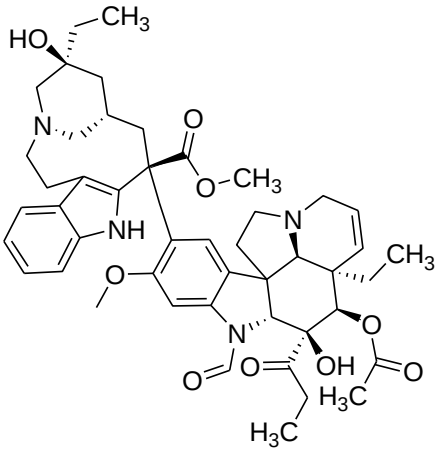


1.2 Reserpine

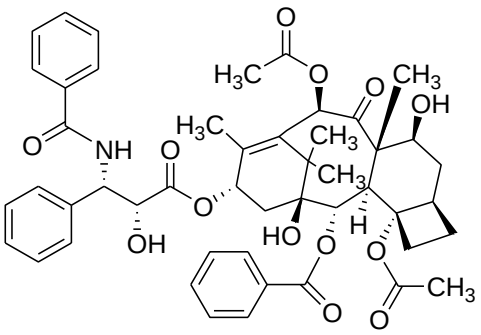
In the last century a series of important medicines were developed from natural products such as morphine (**1.3**) (analgesic) from *Papaver somniferum*, vincristine (**1.4)** (anticancer drug) from *Vinca rosea* and Taxol® (**1.5**) (anticancer drug) from *Taxus brevifolia* (Sarker et al., 2006).



1.3 Morphine



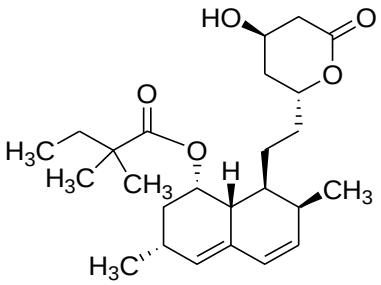
1.4 Vincristine



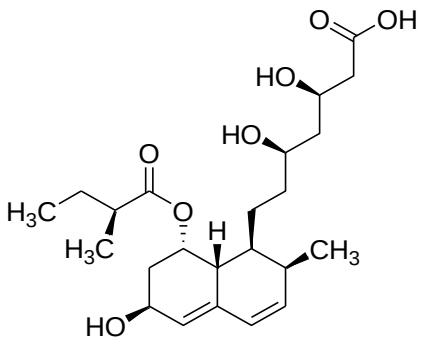
1.5 Taxol®

It is estimated that 40% of modern drugs in use have been developed from natural products (Sarker et al., 2006). More specifically, 39% of the 520 new drugs approved between 1983 and 1994 were natural products or their derivatives and 60–80% of the antibacterial and anticancer drugs were from natural origins (Newman et al., 2003).

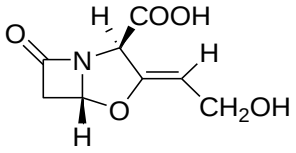
From the 1940s to 2012, of the 175 small molecules used to treat cancer, 47% were natural products or directly derived from natural products (Newman and Cragg, 2012). Eight of the 30 top-selling medicines in 2001, namely simvastatin (**1.6**) (synthetic derivative of a fermentation product of *Aspergillus terreus*), pravastatin (**1.7**) (a derivative of a fungal metabolite from *Penicillium citrinum*), amoxicillin (**1.8**), clavulanic acid (**1.9**) (produced by *Streptomyces clavuligerus*), azithromycin (**1.10**), ceftriaxone (**1.11**), cyclosporin (**1.12**) (isolated from the fungus *Tolypocladium inflatum* (*Beauveria nivea*) and Taxol® (**1.5**) (isolated from the bark of *Taxus brevifolia*) were natural products or their derivatives, together totaling US $16 billion in sales (Newman and Cragg, 2012; Newman et al., 2003; Sarker et al., 2006).



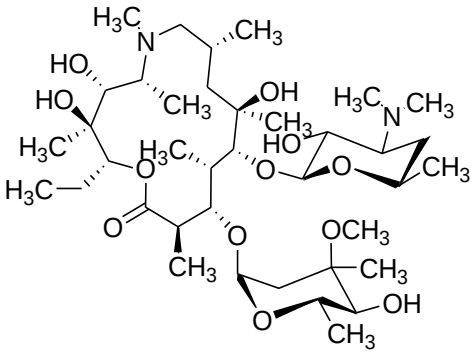
1.6 Simvastatin



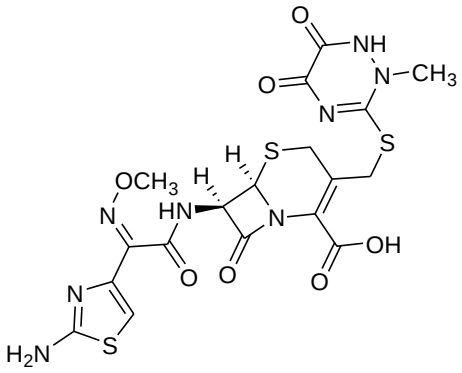
1.7 Pravastatin



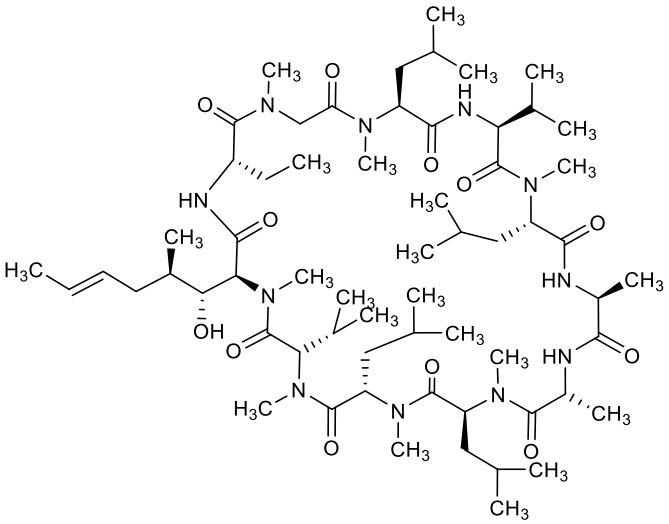
1.9 Clavulanic acid



1.10 Azithromycin



1.11 Ceftriaxone



1.12 Cyclosporin

Figure 1.1: Anti-cancer drugs.

It is estimated that the terrestrial flora consist of between 300000 to 500000 species of higher plants. However, in terms of pharmacological and phytochemical research, it is estimated that only 6% and 15% of the species, respectively, have been investigated.

1.2 DIABETES AND MEDICINAL PLANTS

Diabetes is a major health problem worldwide. Many Southern Africa medicinal plants are used for treatment of diabetes, as will be discussed in Chapter 2. Our hypothesis is that many of the plants that are used by people do have antidiabetic activity and that specific compounds with antidiabetic activity can be isolated from these plants. The aim of this investigation was to investigate the activity and phytochemistry of two plants that are used by people as a treatment for diabetes.

The specific objectives of this project were the:

- Isolation of the secondary metabolites from *H. colchicifolia*.
- Structure elucidation of the compounds isolated from *H. colchicifolia*.
- Antidiabetic activity of the MeOH extract and the isolated compounds from *H. colchicifolia* and *Terminalia sericea*.
- Structure elucidation of isolated compounds from *T. sericea*.

The structure of the rest of the thesis is as follows:

Chapter 2 is a literature review on diabetes, drugs used during treatment and medicinal plants used in South Africa for the treatment of this disease. Chapter 3 gives a brief discussion of the Hypoxidaceae and the phytochemistry of the species of this family. The isolation, purification, identification, and anti-diabetic activity of the MeOH extract and compounds isolated from *Hypoxis colchicifolia* are described. In Chapter 4 the anti-diabetic activity of the MeOH extract from *Terminalia sericea* and the isolation, purification and identification of a compound are discussed.

CHAPTER 2: DIABETES AND MEDICINAL PLANTS: A LITERATURE OVERWIEW

2.1 INTRODUCTION

The International Diabetes Foundation (IDF) define diabetes mellitus, or only diabetes, as "a chronic disease when the pancreas is no longer able to make insulin, or when the body cannot make good use of the insulin it produces''(IDF, 2008). Carbohydrates in food are broken down to glucose in the blood. Insulin, a peptide produced by the beta cells in the pancreas, assists in the uptake of glucose by the cells either for energy or to store it for future uses. Insulin helps in controlling blood glucose levels from being too high (hyperglycaemia) or too low (hypoglycaemia). Over time, high glucose levels in the blood adversely affect a person's health as a result of damage to the heart and blood vessels, kidneys, eyes and nerves.

There are two main types of diabetes:

Type 1 diabetes (insulin-dependent diabetes): Usually as a result of an auto-immune disease, the pancreas is producing very little or no insulin. The disease normally develops in children or young people and is treated by insulin injections.

Type 2 diabetes (non-insulin dependent type diabetes): The body produces enough insulin, but the cells do not utilize it properly. It accounts for about 90% of all cases of diabetes and is closely associated with obesity and lack of exercise.

It is estimated that there are currently 387 million people (8% of the world's population) living with diabetes (half of them undiagnosed) and that this number will increase to 592 million in the next 20 years (IDF, 2008). Diabetes is also a risk factor for active tuberculosis and the reactivation of latent tuberculosis (Leow et al., 2014). The probability of type 1 diabetics to become sick from latent tuberculosis is much higher than that of non-diabetic patients because their immune systems are compromised. In South Africa, the prevalence of diabetes is 8% and according to the most recent report of Stats SA, it is the 5th most important cause of death in South Africa (Witness, 2014). The increase in type 2 diabetes in developing countries, including Africa, is directly associated with inadequate nutrition, obesity and physical inactivity.

2.2 DRUGS USED FOR THE TREATMENT OF DIABETES

The available anti-diabetic drugs can be classified according to their mechanisms (Kemp, 2012; Patel et al., 2012) as summarized in Table 2.1.

Table 2.1: Major drug classes approved for treatment of hyperglycaemia in patients with type 2 diabetes (Kemp, 2012).

Drug class	**Target**	**Action**
Sulfonylureas	K-ATP channel	Insulin secretion
Metformin	Unknown	Hepatic glucose output
Thiazoladinediones	PPARγ	Insulin sensitization
Acarbose	α-Glucosidase	Intestinal glucose absorption
Incretin mimetics	GPLP-1 receptor	GDIS
DPP4 inhibitors	DPP4/ GLPI	GDIS

2.2.1 Sulfonylureas

The sulfonylureas (Figure 2.1) were the first generation of approved antidiabetic drugs developed in 1955, the second-generation sulfonylureas (currently in use) were introduced in 1984 (Kemp, 2012). Even though the first generation drug chlorpropamide (**2.1**) causes side effects more frequently, the incidence of severe hypoglycaemia is high with the second generation glyburide (**2.2**). To correct this problem, Gliclazide® (**2.3**) was developed (Marles and Farnsworth, 1995).

None of the sulfonylureas (first and second generation) currently in use completely normalized insulin secretion. The lack of response to sulfonylureas may be primary (25% - 30% of treated patients) or secondary, occurring in 5% - 10% of patients per year. The side effects of sulfonylureas are hyponatremia, hepatitis, hematological reactions and dermatological effects (Marles and Farnsworth, 1995).

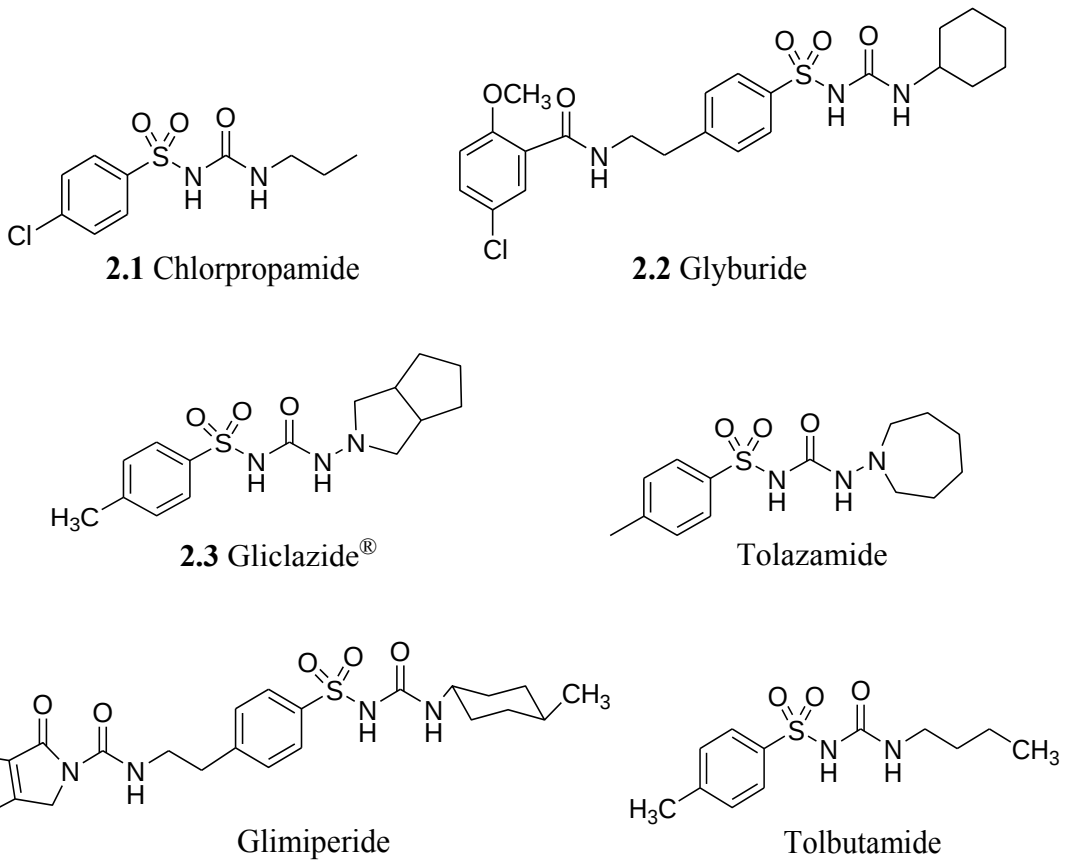
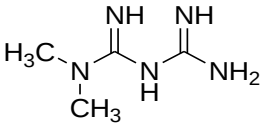


Figure 2.1: Sulfonylurea drugs used for type 2 diabetes.

2.2.2 Metformin

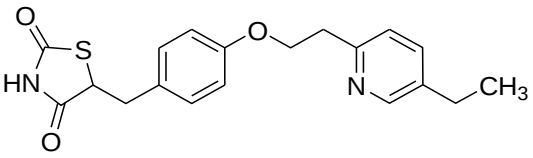
Metformin (**2.4**) is a natural product that was first isolated from *Galega officinalis* (Dunn and Peters, 1995; Witters, 2001). The drug, which was introduced to the market in 1995, is a less toxic biguanides and a potent oral glucose-lowering agent and is the only drug approved to treat diabetes in children (Patel et al., 2012). It is also particularly effective for treatment of overweight and obese people. Metformin (**2.4**) acts by suppression of glucose production by the liver (Kemp, 2012). Metformin (**2.4**) is used for cases of diabetic patients with cardiovascular complication and reduced levels of cholesterol and triglycerides, and has a neutral effect on individuals with normal weight (Kemp, 2012). Metformin (**2.4**) has side effects with the most common adverse effect being gastrointestinal upset. Lactic acidosis can also be a serious problem, particularly in cases of individuals with contra-indications (Kemp, 2012).



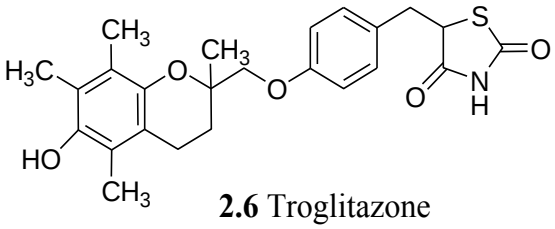
2.4 Metformin

2.2.3 Thiazolidinediones

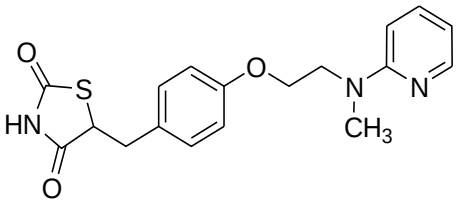
Thiazolidinediones (glitazones) (Figure 2.2) was introduced in 1999. Some of the thiazolidinediones are known for their adverse effects, for example pioglitazone (**2.5**) was suspended from sale in France and Germany due to an increased risk of cardiovascular events and troglitazone (**2.6**) was withdrawn from the market because of the increasing incidence of drug-induced hepatitis (Bernstein, 2012). Because of this, the FDA recommends observation of liver enzymes in the first year of therapy with thiazolidinediones in order to verify possible complications of hepatitis (FDA, 2013).



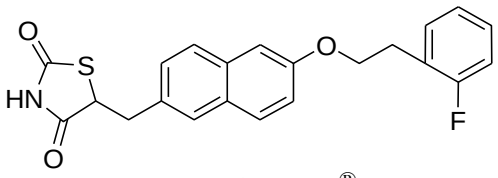
2.5 Pioglitazone



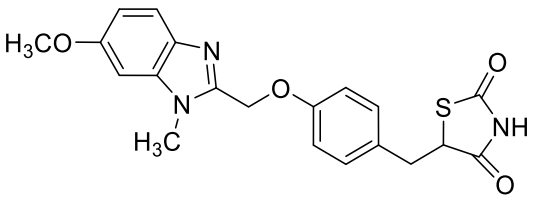
2.6 Troglitazone



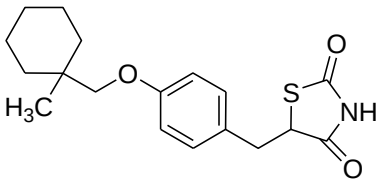
Rosiglitazone



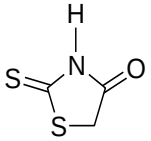
Netoglitazone®



Rivoglitazone



Ciglitazone

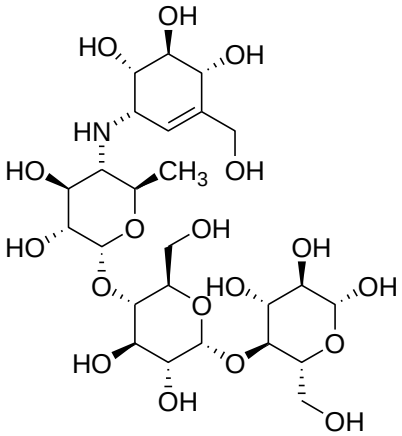


Rodamine

Figure 2.2: Thiazolidinedione drugs used for type 2 diabetes.

2.2.4 Acarbose

Acarbose (**2.7**) is obtained by biotechnology from filtered fungi known as *Actinomycetes*. It is an inhibitor of the enzyme α-glycosidase and is indicated in the treatment of diabetes mellitus type 2 and also type 1 in combination with insulin (Fowler, 2007). It has side effects such as hernias, excessive flatulence and is not recommended for pregnant women, lactation, and bowel disease (Fowler, 2007). Children less than 18 years cannot take this drug (Fowler, 2007).



2.7 Acarbose

2.2.5 Incretin mimetics

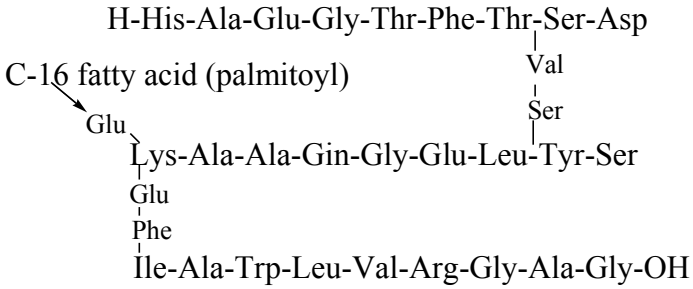
This type of drugs acts by increasing the levels of the incretins, a group of gastrointestinal hormones. In the body, these hormones help to produce more insulin when needed and reduce the amount of glucose being produced by the liver when it is not needed (Wilcox, 2005). It also controls the rate at which the stomach digests food and empties, and appetite. Currently, there are three types of incretin drugs; exenatide (**2.8**), liraglutide (**2.9**) and lixisenstide (**2.10**).

Exenatide (**2.8**), a 39-amino-acid peptide which was approved by the FDA in 2005, is a synthetic version of exendin-4, a hormone found in the saliva of the Gila monster (Kim and Egan, 2008). Exenatide (**2.8**) affects the gastrointestinal system and side effects include acid or sour stomach, diarrhea, belching, indigestion, heartburn, nausea, and vomiting (Kim and Egan, 2008).

H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2

2.8 Exenatide

Liraglutide **2.9** was approved by the FDA in 2010 and may have advantages over current therapies. It acts in a glucose-dependent manner; consequently, it shows lower risk of hypoglycaemia. It can inhibit apoptosis, stimulate the regeneration of beta cells, reduce appetite and assist in maintaining body weight. It also lowers blood triglyceride levels (Kim and Egan, 2008).



2.9 Liraglutide

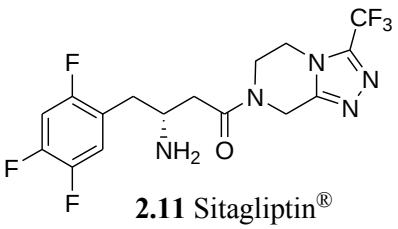
Lixisenatixe (**2.10**) is derived from the first 39 amino acids in the sequence of the peptide exendin-4, found in the Gila monster (*Heloderma suspectum*) and was approved by the FDA in 2013 (Cho et al., 2013).

H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Ser-Lys-Lys-Lys-Lys-Lys-Lys-NH_2

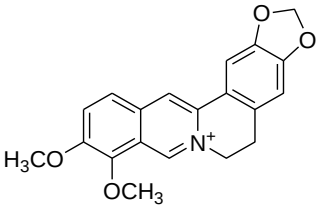
2.10 Lixisenatixe

2.2.6 Dipeptidyl peptidase 4 (DPP4) inhibitors

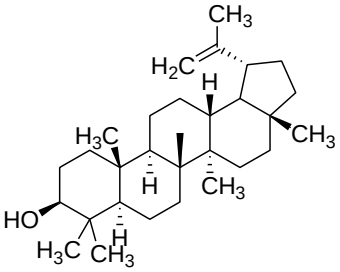
DPP4 inhibitors, known as gliptins, are a new class of oral medication used to treat diabetes mellitus type 2 (Figure 2.3). They were introduced onto the market in 2006 and the first drug of this class approved by the FDA was Sitagliptin® (**2.11**) (Pei, 2012). Side effects such as nasopharyngitis, heart failure, headache, nausea, hypersensitivity and skin reactions have been observed in clinical studies (Cummins, 2012). Although not fully confirmed, there are claims that DPP4 inhibitors increase the risk of pancreas cancer (Nauck and Friedrich, 2013). The natural compounds berberine (**2.12**), belonging to the protoberberine group of isoquinoline alkaloids, and lupeol (**2.13**), a triterpene found in many plants, are also DPP4 inhibitors (Al-masri et al., 2009; Saleem et al., 2014).



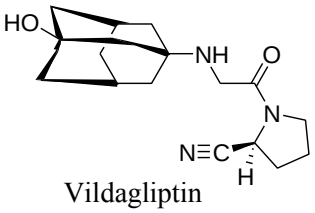
2.11 Sitagliptin®



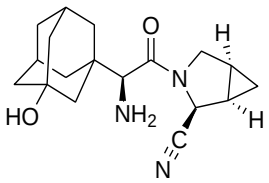
2.12 Berberine



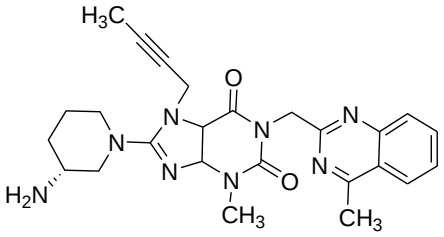
2.13 Lupeol



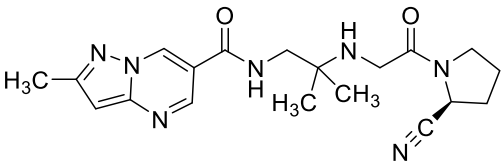
Vildagliptin



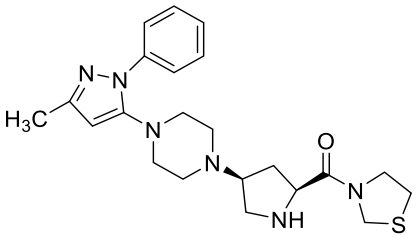
Saxagliptin



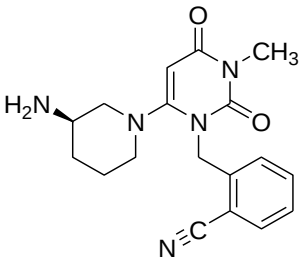
Linagliptin



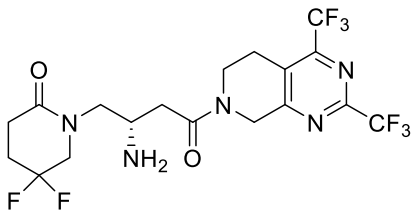
Anagliptin



Teneligliptin



Alogliptin



Gemigliptin

Figure 2.3: DPP4 inhibitor drugs used for type 2 diabetes.

2.3 MEDICINAL PLANTS USED FOR TREATMENT OF DIABETES

To ascertain whether medicinal plants are used for the treatment of diabetes mellitus, the treatment of symptoms of pronounced thirst and polyuria should be considered. In the ethnopharmacological literature, the use of more than 1200 species of organisms to treat symptoms of diabetes have been reported. This represents more than 725 genera in 183 families, extending from marine algae and fungi to advanced plants. Eight hundred plant species may have anti-diabetic potential (Marles and Farnsworth, 1995; Patel et al., 2012). In Table 2.2, the plant families most frequently cited are summarized.

Table 2.2: Plant families most frequently cited for antidiabetic activity (Marles and Farnsworth, 1995).

Family	**Species Cited**	**Total of Species**
Fabaceae	127	18000
Asteraceae	98	21300
Liliaceae	35	6460
Poaceae	30	10000
Euphorbiaceae	30	7000

2.3.1 Medicinal plants used to treat diabetes in South Africa

Plants used in South Africa to treat diabetes have been reviewed by Deutschländer et al., (2009), van der Vender (2008) and Afolayan (2009). Ethnobotanical surveys of plants used for diabetes were recorded by Erastus (2005, Eastern Cape) and Semenya (2012, Limpopo). A summary of these studies are collated in Table 2.3.

Table 2.3: Plants used for diabetes in South Africa.

Family	**Scientific name/ Part used**	**References**
Amaryllidaceae	*Gethyllis namaquensis* (bulb)	(Semenya et al., 2012)
Anacardiaceae	*Lannea edulis* (bark of the woody underground rootstock) *Sclerocarya birrea* (roots, bark,and leaves)	(Deutschländer et al., 2009) (van de Venter et al., 2008)

Apiaceae	*Centella asiatica (roots)*	(Semenya et al., 2012)
	Heteromorpha arborescens (leaves and roots)	(Erasto, 2005)
	Petroselenium crispum (roots)	(Thring and Weitz, 2006)
Apocynaceae	*Catharanthus roseus* (leaves, flowers and twigs)	(Erasto, 2005; van de Venter et al., 2008)
	Hoodia currorii (fleshy stem)	(Von Koenen, 2001)
	Plumeria obtusa (leaves)	(Semenya et al., 2012)
	Vinca major (stems)	(van de Venter et al., 2008)
Araliaceae	*Cussonia spicata* (roots)	(Semenya et al., 2012)
Asphodelaceae	*Aloe ferox* (leaves)	(Deutschländer et al., 2009)
	Bulbine natalensis (roots)	(Erasto, 2005)
	Bulbine frutescens (roots)	(Erasto, 2005)
	Bulbine latifolia (fresh leaves and roots)	(Van Wyk et al., 2005)
	Aloe marlothii (leaves and roots)	(Semenya et al., 2012)
Asteraceae	*Artemisia afra* (leaves an roots)	(Erasto, 2005)
	Brachylaena discolor (leaves)	(Erasto, 2005; van de Venter et al., 2008)
	Brachylaena elliptica (leaves)	(Watt and Breyer-Brandwijk, 1962)
	Brachylaena ilicifolia (leaves)	(Coates Palgrave, 1984)
	Callilepis laureola (roots)	(Semenya et al., 2012)
	Cnicus benedictus (leaves)	(Watt and Breyer-Brandwijk, 1962)
	Conyza scabrida (leaves)	(Thring and Weitz, 2006)
	Elytropappus rhinocerotis (leaves)	(Thring and Weitz, 2006)
	Helichrysum caespititium (whole plant)	(Semenya et al., 2012)
	Helichrysum nudifolium (leaves and roots)	(Erasto, 2005)
	Helichrysum odoratissimum (whole	(Erasto, 2005)

	plant) *Helichrysum petiolare* (whole plant) *Pteronia divaricata* (whole plant) *Schkuhria pinnata* (whole plant) *Tarchonanthus camphoratus* (roots) *Vernonia amygdalina* (leaves) *Vernonia oligocephala* (leaves, twigs, roots)	 (Erasto, 2005) (Deutschländer et al., 2009) (Taylor, 2006) (Semenya et al., 2012) (Erasto, 2005) (Erasto, 2005)
Buddlejaceae	*Chilianthus olearaceus* (leaves and twigs)	(Erasto, 2005)
Cactaceae	*Opuntia ficus-indica* (roots)	(Semenya et al., 2012)
Cannabaceae	*Cannabis sativa* (leaves)	(van de Venter et al., 2008)
Canellaceae	*Warburgia salutaris* (stem bark)	(Deutschländer et al., 2009)
Caricaceae	*Carica papaya* (roots)	(Semenya et al., 2012)
Celastraceae	*Catha edulis* (leaves, stems and roots) *Elaeodendron transvaalense* (bark)	(van de Venter et al., 2008) (Deutschländer et al., 2009)
Combretaceae	*Terminalia sericea* (stem bark and roots)	(Nkobole, 2009)
Cucurbitaceae	*Momordica balsamina* (whole plant) *Momordica charantia* (leaves) *Momordica foetida* (whole plant) *Kedrostis nana* (underground tuber)	(van de Venter et al., 2008) (Semenya et al., 2012) (van de Venter et al., 2008) (Deutschländer et al., 2009)
Ebenaceae	*Euclea undulata* (roots) *Euclea natalensis* (bark and roots)	(Deutschländer et al., 2009) (Deutschländer et al., 2009)
Euphorbiaceae	*Bridelia micrantha* (stem bark) *Ricinus communis* (leaves) *Spirostachys africanus* (bark)	(Deutschländer et al., 2009) (Thring and Weitz, 2006) (Chadwick et al., 2007; Deutschländer et al., 2009)
Fabaceae	*Sutherlandia frutescens* (leaves and	(Chadwick et al., 2007;

	roots)	MacKenzie et al., 2009)
Gentianaceae	*Chironia baccifera* (twigs)	(MacKenzie et al., 2009)
Hypoxidaceae	*Hypoxis hemerocallidea* (corms) *Hypoxis colchicifolia* (corms) *Hypoxis iridifolia* (corms)	(Erasto, 2005; Ojewole, 2006) (Erasto, 2005) (Semenya et al., 2012)
Kirkiaceae	*Kirkia wilmsii* (tuber)	(Semenya et al., 2012)
Lamiaceae	*Leonotis leonurus* (leaves and flowers)	(Thring and Weitz, 2006)
Lauraceae	*Persea americana* (roots)	(Semenya et al., 2012)
Lythraceae	*Punica granatum* (roots)	(Semenya et al., 2012)
Menispermaceae	*Cissampelos capensis* (leaves)	(van de Venter et al., 2008)
Mesembryanthemaceae	*Carpobrotus edulis* (leaves)	(Van Huyssteen, 2003)
Moraceae	*Ficus carica* (roots)	(Semenya et al., 2012)
Moringaceae	*Moringa oleifera* (seeds and leaves)	(Semenya et al., 2012)
Myrtaceae	*Psidium guajava* (leaves and roots) *Syzygium cordatum* (leaves)	(van de Venter et al., 2008) (Musabayane et al., 2005)
Nymphaeaceae	*Nymphaea nouchali (seeds)* *Trigonella foenumgraecum* (seeds)	(Watt and Breyer-Brandwijk, 1962) (Van Wyk B, 1997)
Rhamnaceae	*Ziziphus mucronata* (leaves)	(Mushtaq et al., 2007)
Rubiaceae	*Galium tomentosum* (roots)	(Van Wyk, 2008)
Rutaceae	*Clausena anisata* (roots)	(Ojewole, 2002)
Sapotaceae	*Mimusops zeyheri* (leaves) *Englerophytum magalismontanum* (bark)	(Semenya et al., 2012) (Semenya et al., 2012)
Sterculiaceae	*Hermannia quartiniana* (roots)	(Semenya et al., 2012)
Tiliaceae	*Triumffeta* sp. (roots)	(Semenya et al., 2012)

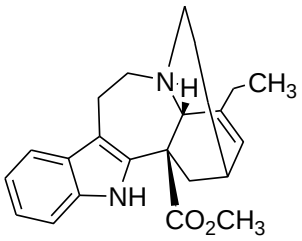
In the Table above, 73 plants used for diabetes in South Africa are listed. However, only a small proportion of these species have been investigated to confirm their antidiabetic activity. The plants subjected to *in vitro* and *in vivo* antidiabetic assays and their active compounds are collated in Table 2.7.

Table 2.4: *In vivo* and *in vitro* studies on antidiabetic plants used in South Africa.

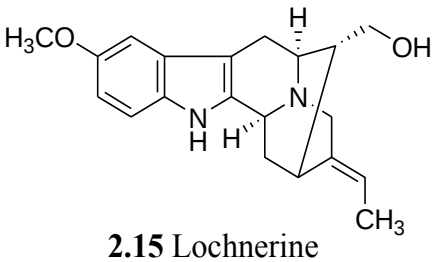
Plant / Family	**Extract/ Part used**	**Assay**	**Activity of the compounds and extracts**
C. edulis (Celastraceae) (van de Venter et al., 2008)	Aqueous (leaves)	Murine cell line C2C12	The extract showed high activity and low toxicity in C2C12 cell line.
C. roseus (Apocynaceae) (Noble et al., 1958.)	Aqueous (leaves) EtOH (leaves)	Animal	Catharanthin (**2.14**), Lochnerine (**2.15**), Tetrahydroalstonine (**2.16**), Vindoline (**2.17**), Vindolinine (**2.18**), Vincamine (**2.19**), Eburnamonine (**2.20**) (Figure 2.4) The compounds show activities, especially vindoline derivatives which have been clinically tested.
H. hemerocallidea (Hypoxidaceae) (Ojewole, 2006)	Aqueous (corms)	Animal	The plant extract (50–800 mg/kg) significantly reduces ($P < 0.05–0.001$) hypoglycaemia in normal (normoglycaemic) and diabetic rats.

L. leonurus (Lamiaceae) (Cunningham, 1988)	Aqueous (leaves)	Animal	The extract cause significant hypoglycaemic effects in rats.
M. foetida (Cucurbitaceae) (van de Venter et al., 2008)	Aqueous (fruits)	Animal Human	Foetidin, mixture 1:1 of β-sitosterol glucoside (**2.21**) and 5,25-stigmasterol glucoside (**2.22**) (Figure 2.5) The extract showed activity, but also toxicity.
M. chararantia (Raman and Lau, 1996)	Aqueous (fruits and seeds)	Animal	Foetidin (**2.21**) , Vicine (**2.23**) (Figure 2.5) The compounds have hypoglycaemic effects, but at high concentrations.
P. guajava (Apocynaceae) (van de Venter et al., 2008)	Aqueous (leaves)	Animal	Ellagic acid (**2.24**) Guiajaverin (**2.25**) Quercitrin (**2.26**) (Figure 2.6) The extract showed activity, but also toxicity in C2C12 cell line.
Syzygium cordatum (Myrtaceae) (Musabayane et al., 2005)	Ethyl acetate (leaves)	Animal	Oleanolic acid (**2.27**) Ursolic acid (**2.28**) Methyl maslinate (**2.29**) Methyl corosolate (**2.30**) (Figure 2.7) The extract decreased plasma glucose in non-diabetic and STZ-diabetic rats. The compounds could be effective in mild

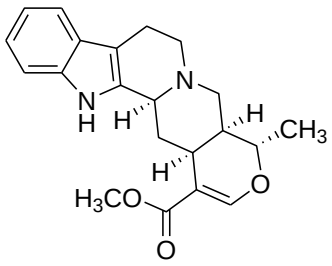
			diabetes mellitus or in cases of glucose tolerance impairment.
Sclerocarya birrea (Anacardiaceae) (van de Venter et al., 2008)	Aqueous DCM:MeOH (1:1)	Murine cell line C2C12	The extract showed high activity and low toxicity in C2C12 cell line.
S. frutescens (Fabaceae)(Chadwick et al., 2007)	Aqueous (leaves)	Animal	The extract shows ability to normalize insulin levels and glucose uptake in peripheral tissues and suppress intestinal glucose uptake.



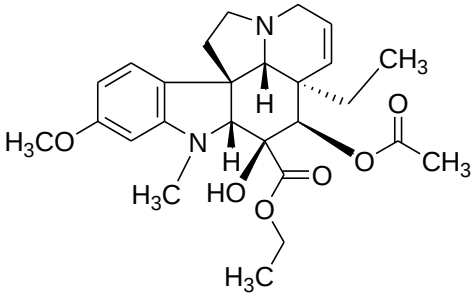
2.14 Catharanthine



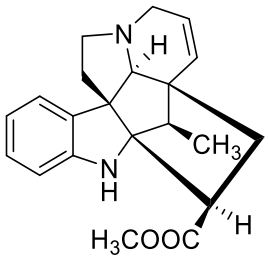
2.15 Lochnerine



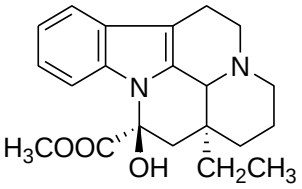
2.16 Tetrahydroalstonine



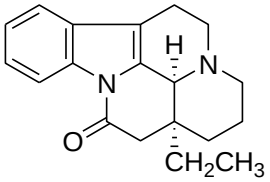
2.17 Vindoline



2.18 Vindolinine

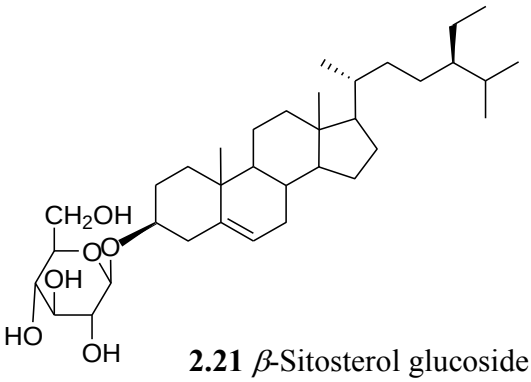


2.19 Vincamine

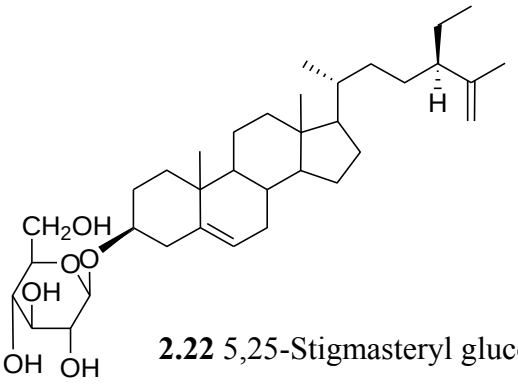


2.20 Eburnamonine

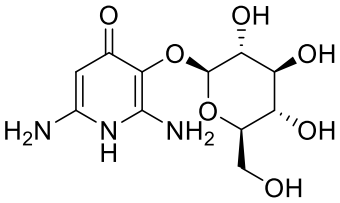
Figure 2.4: Antidiabetic compounds from *Catharanthus roseus*.



2.21 *β*-Sitosterol glucoside

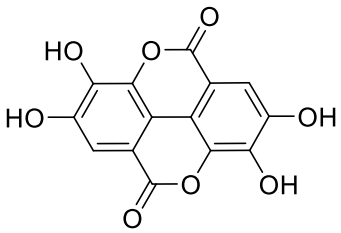


2.22 5,25-Stigmasteryl glucoside

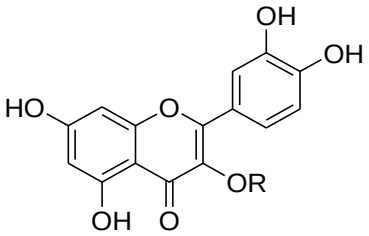


2.23 Vicine

Figure 2.5: Antidiabetic compounds from *Momordica foetida* and *Momordica charantia*.

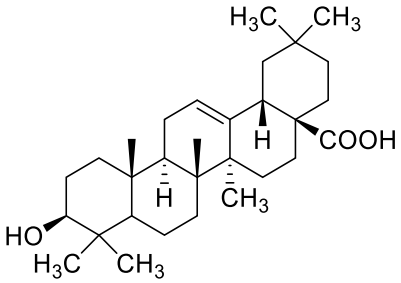


2.24 Ellagic acid

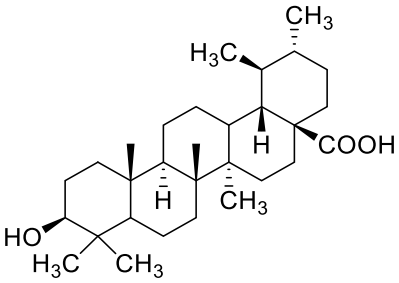


2.25 R= Guiajaverin: L-arabinose
2.26 R= Quercitrin: L-rhamnose

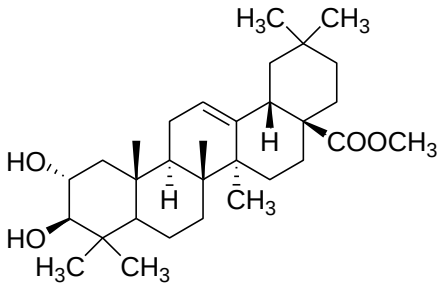
Figure 2.6: Antidiabetic compounds from *Psidium guajava*.



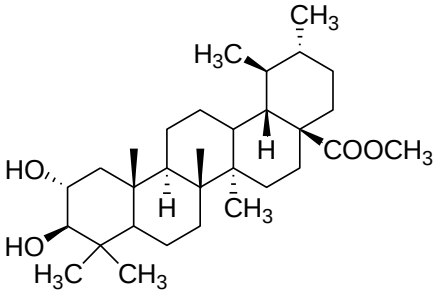
2.27 Oleanolic acid



2.28 Ursolic acid



2.29 Methyl maslinate



2.30 Methyl corosolate

Figure 2.7: Antidiabetic compounds from *Syzygium cordatum*.

2.4 CONCLUSION

It can be concluded that despite the high number of plants used in traditional medicine, only a limited number of plants have been investigated. The validation of traditional knowledge through research on plants used in traditional medicine to treat diabetes can be an effective strategy in combating this disease particularly in Africa where the high cost of drugs available in the market is problematic.

CHAPTER 3: A PHYTOCHEMICAL AND ANTIDIABETIC INVESTIGATION OF *HYPOXIS COLCHICIFOLIA*

3.1 INTRODUCTION AND LITERATURE REVIEW

Hypoxis hemerocallidea Fisch. C.A.Mey. & Avé-Lall (African Potato) is one of the most important medicinal plants in South Africa. The major component of the plant is hypoxoside (**3.1**), a 1,5-diaryl-1-penten-4-yne (Kruger et al., 1994). The related species *Hypoxis colchicifolia* Bak. is also used in traditional medicine. One of the medicinal uses of *H. colchicifolia* is for the treatment of diabetes. This work describes the composition and antidiabetic activity of the MeOH extract of *H. colchicifolia*.

While writing this thesis (December 2014), a paper was published in which Bassey et al. (2014) reported the isolation of two new phenolic glycosides, 3-hydroxy-4-*O*-β-D-glucopyranosylbenzaldehyde and 1,5-bis(3,4-dihydroxyphenyl)-1,2-dihydroxy-4-pentyne-2-*p*-coumaroyl-β-D-gluco-pyranoside from the corms of *H. colchicifolia*. They also identified the norlignan glycosides hypoxoside, dehydroxyhypoxoside and bis-dehydroxyhypoxoside.

3.1.1 The Hypoxidaceae family

Hypoxidaceae is a family of flowering plants, grouped in the order Asparagales of the Monocotyledons. The family consists of probably more than six genera with about 100-200 species (List, 2013). The family members are small to medium herbs, with grass-like leaves and an invisible stem, modified into branches. Hypoxidaceae are herbaceous perennial herbs with corms or rhizomes, some have tubers. The flowers are born on leafless shoots, with three article fittings, radially symmetric with six yellow petals (Hutchings, 1996; Leistner, 2000).

1.2 The genus *Hypoxis*

The name *Hypoxis* is derived from Greek with ‘hypo’ meaning ‘below’ and ‘oxy’ referring to the base ends of the ovary or fruit. The *Hypoxis* genus was established by Linnaeus in 1759 and then alternately assigned within and outside the family Amaryllidaceae (Monocotyledons). In 1964 it was moved to the monophylitic family Hypoxidaceae (Guide to the flora of the Carolina, 2005). There are 96 known species of *Hypoxis* in Africa, 16 in North and South America, and 7 in Southeast Asia and Australia.

3.1.3 Phytochemistry of Hypoxidaceae

Norlignans are the characteristic class of secondary metabolites isolated from *Hypoxis*. The chemical structures of norlignans are composed of phenylpropane (C6-C3) and phenylethane (C6-C2) units (Suzuki and Umezawa, 2007). Based on the linkage position between the two units, they are classified into three groups: norlignans from conifers with C7-C8′ linkage; norlignans from monocotyledons with a C8-C8′ linkage; norlignans from other plant sources with C8-C9′ linkage (Suzuki and Umezawa, 2007). Recently, Suzuki and Umezawa (2007) proposed biosynthetic pathways for the formation of three groups of norlignans from phenylpropanoid monomers via *p*-coumaryl *p*-coumarate as a key intermediate (Figure 3.1).

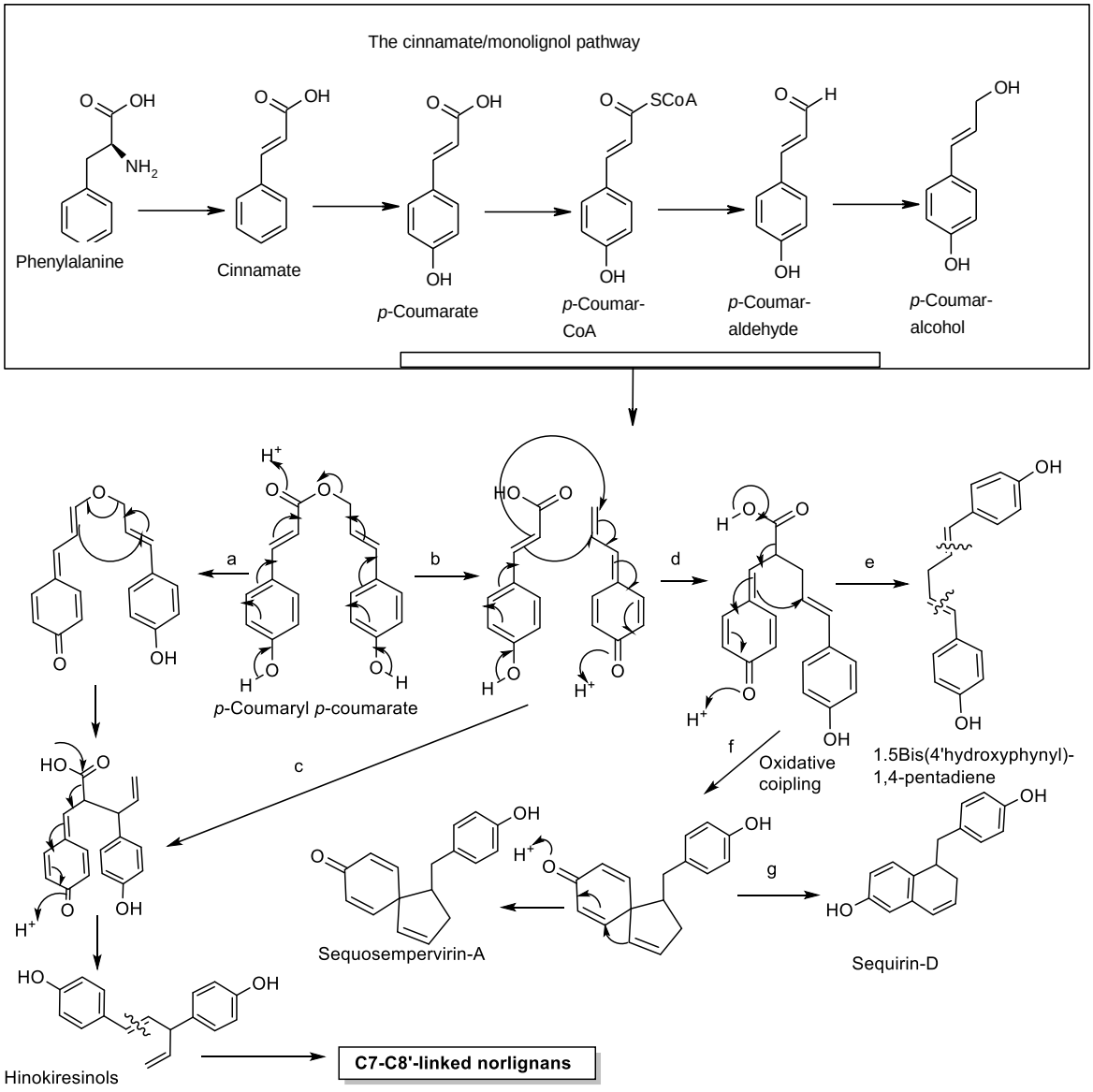
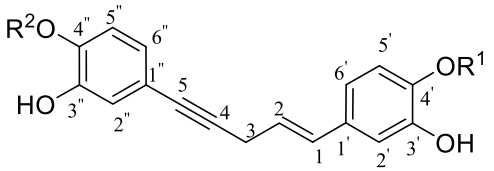


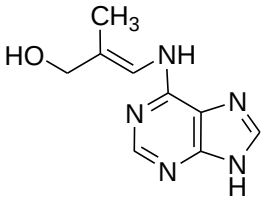
Figure 3.1: Biosynthetic scheme for the norlignans.

In 1982 Marini-Bettolo et al. isolated an unsaturated norlignan glycoside from the rhizomes of *H. obtusa* collected in Mozambique. It was the first compound identified from the Hypoxidaceae and therefore named hypoxoside (**3.1**), ((*E*)-1,5-bis[(3'-hydroxy-4'-O-β-D-glucopyranosyloxy)-phenyl]-1-penten-4-yne) (Marini-Bettolo et al., 1982). The isolation of this compound was also reported from *H. hemerocallidea* and *H. nyasica* (Drewes et al., 1983; Galeffi and Multari, 1987).

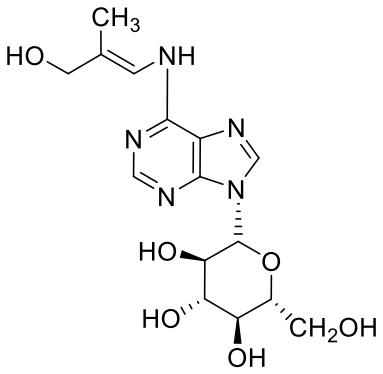
The other compounds isolated from the various *Hypoxis* species are obtuside A (**3.2**), obtuside B (**3.3**), zeatin (**3.4**), zeatin glucoside (**3.5**), obtusaside (**3.6**) from the *H. obtusa* (Msonthi et al., 1990); acuminoside (**3.7**) from *H. acuminata*; mononyasine A (**3.8**), mononyasine B (**3.9**), nyaside (**3.10**) (Marini-Bettolo et al., 1991) and nyasicoside (**3.11**) (Messana et al., 1989) from *H. nyasica*; interjectin (**3.12**) from *H. interjecta* and *H. multiceps* (Marini-Bettolo et al., 1991) and β-sitosterol (**3.13**) from the corms of *H. hemerocallidea*, popularly known as the 'African Potato' (Appleton et al., 2012).



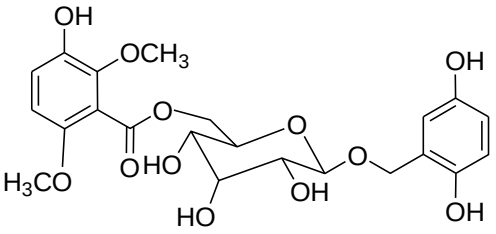
3.1 $R^1 = R^2 =$ Glu: Hypoxoside
3.2 R^1 = Glu, R^2 = H: Obtuside A
3.3 R^1=H, R^2 = Glu: Obtuside B



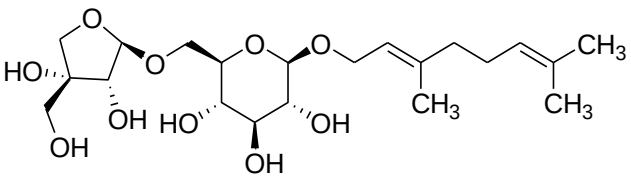
3.4 Zeatin



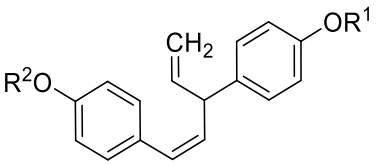
3.5 Zeatin glucoside



3.6 Obtusaside



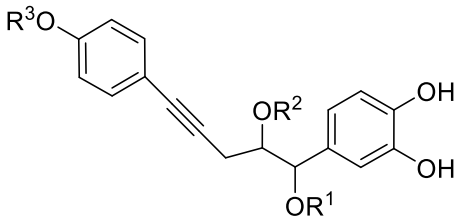
3.7 Acuminoside



3.8 R^1=Glu, R^2=H: Mononyasine A

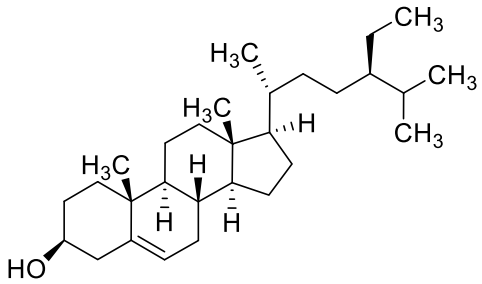
3.9 R^2=Glu, R^1=H: Mononyasine B

3.10 R^1=Apiose-Glu, R^2=Glu: Nyaside



3.11 R^1= R^3=H, R^2=Glu: Nyasicoside

3.12 R^1=H, R^2=*p*-coumaroyl-Glu, R^3=Glu: Interjectin



3.13 β-Sitosterol

Figure 3.2: Compounds isolated from *Hypoxis*.

3.1.4 Medicinal uses and biological activity of *Hypoxis* species

According to Appleton (2004), eleven species of the genus *Hypoxis* are used for traditional purposes throughout southern Africa. However, a survey of the most popular medicinal plants marketed in South Africa showed that only two species, *H. hemerocallidea* and *H. colchicifolia*, are routinely sold, especially on the markets in KwaZulu-Natal (Cunningham, 1988). The high quantities of these species marketed in Southern Africa put them under threat of extinction.

The corm of *H. hemerocallidea* (*H. rooperii*) known as African potato, is widely used in traditional African medicine for the treatment of many general ailments: infusions are used as antiemetics, to treat dizziness, bladder disturbances, insanity (Verschaeve and Van Staden, 2008), wounds and burns (Eloff et al., 2008), allergies, ulcers, arthritis, hypercholesterolaemia and infertility (Mills et al., 2005). The juice is used to treat burns, prostate problems, testicular tumours, benign prostatic hyperplasia (BPH) and urinary tract infection (Drewes et al., 2008; Verschaeve and Van Staden, 2008). Plant decoctions have purging effects (Aremu et al., 2010).

The aqueous extracts of African Potato have anti-inflammatory, anti-diabetic and antinociceptive properties in rats and mice (Ojewole, 2006). Gaidamashvili and van Staden (2002) reported that the ethanolic extracts of fresh leaves of *H. hemerocallidea* is active against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* with MIC $\leq$ 0.63 mg mL^{-1}.

The aqueous extract (corms) of *H. colchicifolia* (Figure 3.3) is used to treat diabetes (Erasto, 2005), HIV, inflammatory conditions, impotence and sterility, hysterics and is used as a tonic and an emetic (Ncube et al., 2013). Nair also reported its use for heart problems, impotency, female infertility, insanity and as a vermin killer (Nair, 2006).

Figure 3.3: *H. colchicifolia* corms.

H. colchicifolia (leaves) had good COX-2 (cyclooxygenase) inhibitory activity (Aremu et al., 2010). The aqueous and ethanolic extracts of *H. colchicifolia* leaves were found to inhibit angiotensin converting enzyme (ACE) at a concentration of 333.3 ng mL^{-1} (Nair, 2006).

3.1.5 *Hypoxis colchicifolia*

H. colchicifolia (*H. latifolia*) (Figure 3.4) is after *H. hemerocallidea* the second most important *Hypoxis* medicinal species with commercial value in South Africa. Although it is widely used in traditional medicine, the main secondary metabolites were not identified until now.* One of the medicinal uses of *H. colchicifolia* is for the treatment of diabetes (Appleton et al., 2012).

The plants grow in grassland areas with a height of 25-60 cm and have big underground tubers that allow it to survive the constant grass fires common to this vegetation type. The leaves are long and erect, are stiff and almost hairless. The leaves die during the winter months and the new flowers appear after the early spring rains from the apex of the rhizome.

*While writing this thesis (December 2014), a paper on the chemistry of *H. colchicifolia* was published (Basset et al., 2014).

Figure 3.4: *H. colchicifolia*.

H. colchicifolia occurs widespread from the eastern coast to the interior of southern Africa and occurs in the Eastern Cape and KwaZulu-Natal provinces of South Africa (Van Wyk et al., 2005). It is found on sandy or poor soils in grassland.

3.2 RESULTS AND DISCUSSION

In a previous investigation in our research group it was shown that the crude methanol extracts of both *H. hemerocallidea* and *H. colchicifolia* lowered the blood glucose level of STZ-induced rats and, therefore, that these two plants have antidiabetic activity (Thring and Weitz, 2006).

The phytochemistry of *H. hemerocallidea* (syn. *H. rooperi*) has been investigated previously (Drewes et al., 2008; Drewes et al., 1983; Drewes et al., 1989; Kruger et al., 1994; Laporta et al., 2007). When this project was initiated, nothing had been published on the phytochemistry of *H. colchicifolia* and therefore it was decided to investigate the phytochemistry and antidiabetic activity of *H. colchicifolia.* While writing this thesis (December 2014), a paper on the chemistry of *H. colchicifolia* was published (Bassey et al., 2014).

3.2.1 Isolation of compounds by preparative HPLC

HPLC was used for the analysis and fractionation of the MeOH extract of *H. colchicifolia* corms. In the chromatogram obtained on a semi-preparative column (Figure 3.14), four peaks were observed. Three of the peaks (P2 - P4) had closely related retention times. By using the same HPLC method, the MeOH extract was separated into four fractions (F1 – F4) corresponding to the four peaks (P1 – P4) in the chromatogram.

NMR analysis of F1 (Figure 3.15) showed that it consisted of a small amount of complex tannins and carbohydrates and this fraction was not investigated further. Fraction F2 (Figure 3.16) consisted of a single compound, hypoxoside (**3.1**), which was first isolated from *H. nyasica* (Marini-Bettolo et al., 1982) and then from *H. hemerocallidea* (Drewes et al., 1983). Fraction F3 consisted of a mixture of two deoxy analogues of hypoxoside named dehydroxyhypoxoside A (**3.14**) and dehydroxyhypoxoside B (**3.15**) in 3:1 ratio. Fraction F4 consisted of a single compound, bis-dehydroxyhypoxoside (**3.16**).

A contaminant **3.17**, widely used in industry as an intermediate for the production of epoxy resins (Saiyood et al., 2010), was isolated from the ethyl acetate-soluble material of the methanol extract.

Although the isolation of **3.15** and **3.16** have been described in literature (Bassey et al., 2014; Kruger et al., 1994; Laporta et al., 2007), there are no NMR data for these compounds in the literature. The authors in these three papers based the structures of **3.15** and **3.16** on LC-MS data. Compound **3.14** has not yet been reported. Herewith, the NMR spectra of **3.1** and **3.14 – 3.16** are assigned.

3.2.2 Hypoxoside (3.1)

Fraction F2 consists of a single compound, which was identified as hypoxoside (**3.1**). In the HPLC chromatogram, the retention time of F2 was the same as that of the major peak in the methanol extract of *H. hemerocallidea*. The structural elucidation of **3.1** isolated by Marini-Betollo et al. and Drewes et al. was based on NMR studies of the peracetylated compound (Drewes et al., 1983; Marini-Bettolo et al., 1982). In the literature, there is only a single report on the NMR assignment of the free phenolic **3.1** (Laporta et al., 2007). Upon careful inspection, we have observed several discrepancies in the NMR assignments by Laporta et al. and since the structures of derivatives of **3.1** are based on the NMR data of **3.1**, it is important that the assignments of the NMR spectra of this compound should be unambiguous. Therefore, we have reinvestigated the NMR assignments of **3.1**.

The ^{1}H and ^{13}C NMR spectra of **3.1** are given in Plate 1a and Plate 1b, respectively. The assignment of the spectra was based on interpretation of the DEPT-135, $^{1}H,^{1}H$-COSY, $^{1}H,^{13}C$-HSQC and $^{1}H,^{13}C$-HMBC spectra. In the ^{1}H NMR spectrum, the resonances for the five-carbon backbone of **3.1** were observed at δ_H 6.57 (br d, J = 15.7 Hz, H-1) and δ_H 6.13 (dt,

J = 15.7 and 5.7 Hz, H-2) for the *trans*-alkene and δ_H 3.28 (dd, J = 5.7 and 1.5 Hz) for H-3. The resonance of H-3 was partly obscured by the solvent peak. The corresponding ^{13}C NMR resonances were observed at 131.8 (C-1), 124.5 (C-2), 23.3 (C-3), 86.4 (C-4) and 83.3 (C-5). C-1, 2 and 3 were assigned from the $^1J_{CH}$ correlations with H-1, 2 and 3 respectively, observed in the HSQC spectrum. The two acetylenic carbons C-4 and C-5 was assigned based on the $^3J_{CH}$ correlations with H-2 and H-3, respectively, in the HMBC spectrum.

In hypoxoside (**3.1**), the two aryl rings attached to C-1 and C-5 have the same substitution pattern and the assignment of resonances to a specific ring was not crucial for the structural elucidation of **3.1**. However, to assist with the structural elucidation of analogues of hypoxoside (**3.1**) where the two rings have different substitution patterns, it is important that the assignment of the resonances to the two rings of **3.1** must be unambiguous.

Both rings display the three aromatic protons as ABX spin systems. The resonances of H-5' and H-5", which have only *ortho* coupling, overlap and are observed as a two-proton doublet (J = 8.3) at δ_H 7.12. To differentiate between H-2' / H-6' and H-2" / H-6", the correlations observed in the HMBC spectrum were crucial. The resonances at δ_H 6.92 (dd, J = 2.0 Hz) and δ_H 6.83 (dd, J = 8.3 and 2.0 Hz) both correlated to the resonance at δ_C 131.8 (C-1) and were assigned to H-2' and H-6', respectively. Likewise, the resonances at δ_H 6.90 (d, J = 2.0 Hz) and δ_H 6.87 (dd, J = 8.3 and 2.0 Hz) correlated both to the resonance at δ_C 83.3 (C-5) and were assigned to H-2" and 6", respectively.

The two anomeric protons of the glucose moieties were observed at δ_H 4.79 and 4.75 (d, J = 7.5 Hz). The linkages through oxygen of the sugars moieties and C-4' and C-4" of the aromatics rings were derived from the HMBC spectrum (Plate 1e), where the anomeric protons (H-1'" and H-1"", δ_H 4.79 and 4.75) correlated with C-4' and C-4" (δ_C 146.9 and 146.3). The assignments of the methine carbohydrate carbons were also based on the correlations observed in the HMBC spectrum. For each of the glucosyl carbons, separate sets of resonances were observed for the two carbohydrate moieties in the ^{13}C NMR spectrum; however, the chemical shifts were so close to each other that the resonances could not be assigned to the individual sugar moieties.

The long-range correlations observed for **3.1** are illustrated in Figure 3.5. The NMR data of **3.1** are summarized in Table 3.1.

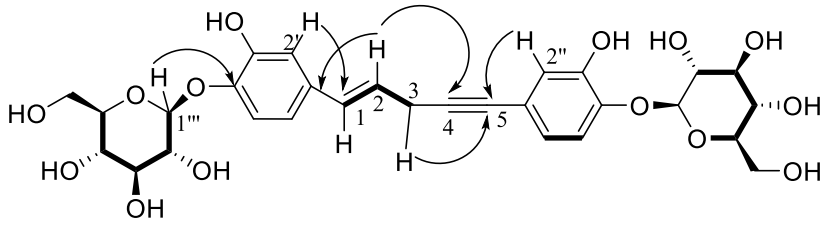


Figure 3.5: Key HMBC ($^{1}H \rightarrow ^{13}C$, arrow) and key $^{1}H-^{1}H$ COSY (bold) correlations for **3.1**.

Analysis of **3.1** by high-resolution mass spectrometry (Plate 1h) using electrospray ionization in the negative mode show a peak at *m/z* 605.1868 $[M-H]^-$, which correlated with the molecular formula of $C_{29}H_{34}O_{14}$. Additional peaks in the negative mode were observed at *m/z* 641.2382, corresponding to a chloride adduct $[M+Cl]^-$, $C_{29}H_{34}O_{14}Cl$, and a peak at *m/z* 283.13 ($C_{17}H_{12}O_4$). The last peak indicated the loss of two glucosyl moieties to form the aglycone.

The IR spectrum revealed absorption bands at 780 and 860 cm^{-1} suggesting C-H rocking vibrations. The C-H in-plane bending is shown by the stretching vibration band at 2894 cm^{-1}. The absorption bands at 1246 and 3332 cm^{-1} denote OH bending and stretching, respectively. The bands at 896 and 1014 cm^{-1} suggest the C-H out-of-plane deformation when attached to a carbon by a double bond (C=C-H). The C=C in an aromatic ring is shown by two bands of medium intensity at 1510 and 1587 cm^{-1} (Nair, 2006).

The UV spectrum was obtained from the diode-array detection of **3.1** in the HPLC. The UV spectrum of **3.1** shows three absorption bands respectively at λ 210 (maximum absorption), 257 and 291 nm. The same absorption profile was found by Kruger and Laporta, confirming the identity of the compound (Kruger et al., 1994; Laporta et al., 2007).

Table 3.1: ^{1}H and ^{13}C NMR data of hypoxoside **3.1** in CD_3OD (*J* in Hz).

Position			Assignment by Laporta et al 2007	
	δ_H	δ_C	δ_H [$(CD_3)_2CO$]	δ_C (CD_3OH)
1	6.57, d (15.7)	131.8	6.51	131.6
2	6.13, dt (15.7, 5.7)	124.5	6.08 (15.7, 5.5)	124.3
3	3.28, dd, (5.7, 1.5)	23.3	3.23	23.2
4	-	83.3	-	86.2
5	-	86.3	-	83.2
1'	-	134.6	-	114.4
2'	6.92, d (2.0)	114.5	7.08	118.2
3'	-	148.5, 148.1	-	148.3, 148.0
4'	-	146.9 or 146.3	-	146.8; 146.2
5'	7.12, d (8.3)	118.9 or 118.5	6.85	120.0
6'	6.83, dd (7.0, 1.5)	119.4	6.82	124.5
1"	-	120.3	-	134.3
2"	6.90, d (2.0)	120.1	7.06	118.5
3"	-	148.5, 148.1	-	148.3, 148.0
4"	-	146.9 or 146.3	-	146.8; 146.2
5"	7.12, d (8.3)	118.9 or 118.5	6.87	114.3
6"	6.87, dd (8.3, 2.0)	124.7	6.77	119.3
1'''/1''''	4.79 or 4.75, d (7.5)	104.4 or 104.0	4.74; 4.72, d (7.2)	104.1; 103.7
2'''/2''''	3.52-3.44, m	74.9 or 74.8	3.40–3.37, m	78.2
3'''/3''''	3.52-3.44, m	77.64 or 77.60	3.48–3.42, m	74.8
4'''/4''''	3.50-3.41, m	71.3	3.40–3.37, m	71.2
5'''/5''''	3.50-3.41, m	78.4, 78.3	3.48–3.42, m	77.6
6'''/6''''	3.90, 3.72, br d (12.2)	62.4	3.85; 3.69, d (12)	62.3

3.2.4 Bis-dehydroxyhypoxoside (3.16)

The MS data of the three major compounds observed in the HPLC indicated that they are hypoxoside (**3.1**) (R_t 13.072), deoxy derivative(s) of hypoxoside (R_t 13.655) and a dideoxy derivative of hypoxoside (R_t 14.339). NMR data indicated that the dideoxy derivative **3.16** has two identical aryl rings on C-1 and C-5. Since the NMR data of **3.16** was used to assign the structures of the deoxy-derivatives, the NMR assignment of **3.16** will be discussed first.

Compound **3.16** was obtained as a brown amorphous solid. The high-resolution mass spectrum of **3.16** (Plate 3h) using electrospray ionization in the positive mode showed a peak at *m/z* 597.1950 $[M+Na]^+$ corresponding to a molecular formula of $C_{29}H_{34}O_{12}$ (that indicated 13 indices of hydrogen deficiency). In the negative mode peaks were observed at *m/z* 609.2321 $[M+Cl]^-$, $C_{29}H_{34}O_{14}Cl$, corresponding to a chloride adduct, and *m/z* 411.1911 ($C_{23}H_{23}O_7$) and 249.1205 ($C_{17}H_{13}O_2$), indicating the loss of one and two glucosyl moieties, respectively, to form the aglycone (Bassey et al., 2014).

Although the structure of **3.16** was proposed by Kruger et al. (1994), Laporta et al. (2007), and Bassey et al. (2014), these authors only reported MS data and the NMR of **3.16** was reported . Herewith we describe the first NMR assignment of **3.16**.

The NMR profile for the 5-carbon backbone (C-1 - C-5) of **3.16** is similar to that of **3.1**. Of importance are the unambiguous assignments of C-1 and C-5 in the ^{13}C NMR spectrum. In the spectrum of **3.16** the assigned to H-1 (δ_H 6.58, d, J =15.8 Hz) correlated with the ^{13}C resonance at δ_C 131.7, which was assigned to C-1. To differentiate between the two acetylenic carbon resonances, the correlation in the HMBC spectrum of H-2 (6.17, dt, J = 15.8, 5.8 Hz) to the carbon resonance at δ_C 86.5 was used to assign this resonance to C-4. Therefore, the resonance at δ_C 83.5 was assigned to C-5.

Further analysis of the ^1H and ^{13}C NMR spectra (Plate 3a and Plate 3b, respectively) revealed the presence of two *para*-substituted aryl rings. In the ^1H NMR spectrum, the resonances of H-3',5' and H-3",5" had the same chemical shift at δ_H 7.04 and a 4-proton doublet (J = 9.1 Hz) was observed. However, there was a slight difference in chemical shift for the resonances of H-2',6' and H-2",6" at δ_H 7.04. The assignment of the resonances was based on correlations observed in the HMBC spectrum. The two-proton doublet at δ_H 7.37 (J = 9.0 Hz) correlated with the carbon at δ_C 82.0 (C-5) and could be assigned to C-2",6". Likewise the two-proton

doublet at δ_H 7.33 correlated with the resonance at δ_C 130.4 (C-1) and could be assigned to C-2', 6'.

The linkages between the aromatic ring and sugar moieties at C-4' and C-4" were derived from the HMBC spectrum (Plate 3d) in which the anomeric protons H-1'",1"" (δ_H 4.91) were correlated to the phenyl C-4' and C4" at δ_C 158.7, 158.5.

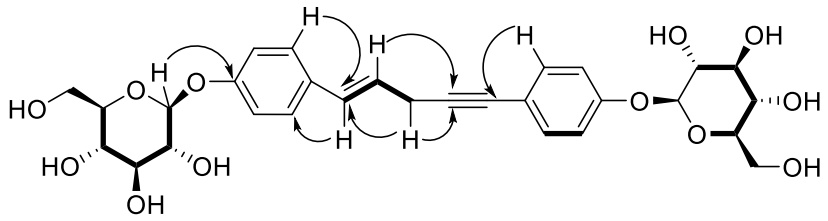


Figure 3.6: Key HMBC ($^1H \rightarrow ^{13}C$, arrow) and key $^1H-^1H$ COSY (bold) correlations for **3.16**.

As can be expected, the IR spectrum of **3.16** (Plate 3f) is same as that of **3.1** and revealed absorption bands at 780 and 860 cm^{-1} suggesting C-H rocking vibrations. The C-H in-plane bending is shown by the stretching vibration band at 2894 cm^{-1}. The absorption bands at 1246 and 3332 cm^{-1} denote OH bending and stretching respectively. The bands at 896 and 1014 cm^{-1} suggest the C-H out-of-plane deformation when attached to a carbon by a double bond (C=C-H). The C=C in an aromatic ring is shown by two bands of medium intensity at 1510 and 1587 cm^{-1}.

The UV spectrum of **3.16** (Plate 3g) showed two absorption bands respectively at 210 (maximum absorption) and 259 nm. This spectrum differs from **3.1** and **3.14** because the band at 300 nm does not appear. This is contrary to the UV spectrum presented by Kruger (1994) and also by Laporta (2007) for this compound.

3.2.3 Dehydroxyhypoxoside A (3.14) and B (3.15)

HPLC analysis of fraction 3, obtained by preparative HPLC separation of the crude extract, showed the presence of two compounds, a major and a minor compound. Initially it was assumed that the fraction was contaminated with **3.16** (R_t 14.339), but after careful consideration of the retention times it was concluded that the second (minor) peak with R_t = 14.26 was indeed a different compound from **3.16**. We were not able to separate these compounds. The compounds were identified as two deoxy derivatives of hypoxoside which

we named dehydroxyhypoxoside A (**3.14**) and dehydroxyhypoxoside B (**3.15**) and they were isolated as a mixture in a 1:2 ratio of these compounds.

The mixture of the compounds **3.14** and **3.15** was obtained as a brown, amorphous solid. The high-resolution MS using electrospray ionization in the negative mode showed a peak at *m/z* 589.1915 $[M-H]^-$ corresponding to a molecular formula of $C_{29}H_{33}O_{13}$ (that indicate 13 indices of hydrogen deficiency). Peaks at *m/z* 625.2342 corresponding to $[M+Cl]^-$ $C_{29}H_{34}O_{13}Cl$ (a chlorine adduct), *m/z* 427.1825 ($C_{23}H_{23}O_8$) and 265.1147 ($C_{17}H_{12}O_3$) were also observed. The later peaks indicated the loss of one and two glucosyl moieties, respectively, to form the aglycone.

The presence of **3.14** (refer to as dehydroxyhypoxoside) in *H. hemerocallidea* has been reported by Kruger et al. and Laporta et al., whereas Bassey et al. described the isolation of **3.14** from *H. colchicifolia* (Bassey et al., 2014; Kruger et al., 1994; Laporta et al., 2007). However, in all publications only MS data were provided and no NMR data were reported.

The ^1H and ^{13}C NMR spectra showed that fraction 3 consisted of a 2:1 mixture of compounds. Both the major and the compounds had structural elements in common with **3.1** and **3.16**. In the ^1H NMR spectra, a slight but clearly discernible chemical shift difference was observed for H-1 of **3.1** (δ_H 6.57) and **3.16** (δ_H 6.64). In the spectrum of fraction 3, the major component (**3.15**) had a peak for H-1 at δ_H 6.58, indicating that the A-ring had most likely the same substitution pattern as hypoxoside (**3.1**), whereas H-1 for the minor component (**3.14**) resonated at δ_H 6.64, which is in agreement with an A-ring with a substitution pattern similar to that of bisdehydroxyhypoxoside (**3.16**). Analysis of the aromatic region of the ^1H NMR spectrum confirmed that the mixture consists of two isomeric compounds, the major isomer **3.15** with an 1,3,4-trisubstituted A-ring and a 1,4-disubststituted B-ring and the minor isomer **3.14** with a 1,4-disubstituted A-ring and a 1,3,4-trisubstitued B-ring.

The key to determine on which ring the OH was, was the analysis of the HMBC (Plate 2h) spectrum in which it was possible to establish correlations between H-2' and C-1 for compound **3.14** and H-2" and C-5 for compound **3.15**.

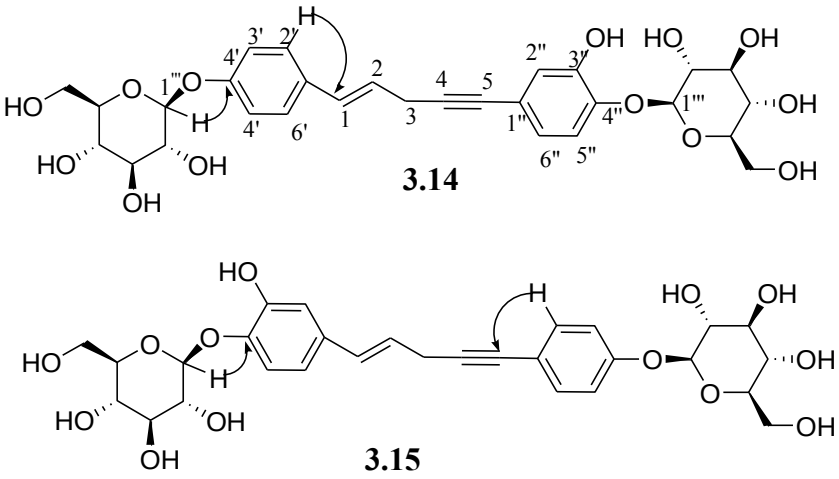
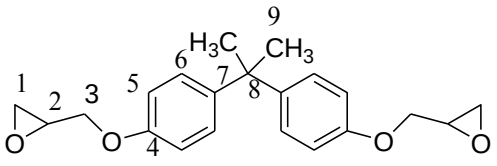


Figure 3.7: Key HMBC ($^{1}H \rightarrow ^{13}C$, arrow) correlations for isomers **3.14** and **3.15**.

The IR spectrum of the compounds **3.14** and **3.15** has the same profile as described for **3.1** and **3.16**. The UV spectrum of **3.14** and **3.15** shows three absorption bands at 210 (maximum absorption), 258 and 290 nm. The same UV profile was reported by Kruger (1994) and Laporta (2007).

3.2.4 Bisphenol A diglycidyl ether (3.17)

The structural determination of **3.17** was achieved through a combination of different spectroscopic techniques including MS, ^{1}H, ^{13}C, COSY, HMBC and HSQC NMR experiments.



3.17

The analysis of the COSY (Plate 4c) and ^{1}H NMR spectra (Plate 4a) showed the presence of a *para*-substituted aromatic ring at δ_H 6.89 and δ_H 7.13. The COSY spectrum allowed us to observe the correlation between H-5 (δ_H 7.13, d, J = 9.0 Hz) and H-6 (δ_H 6.81, d J = 8.9 Hz). The HSQC spectrum (Plate 4d) showed that in the aromatic ring C-5 (δ_C 157.4) correlates to H-5 at δ_H 7.13, and C-6 (δ_C 114.3) was attached to H-6 at δ_H 6.83.

A *gem*-dimethyl group was observed at δ_H 1.64 (6H, s, H-9) (Plate 4a), and HMBC showed the correlation between H-9 and C-8 (δ_C 41.7) and C-7 (δ_C 143.4). In the NMR spectrum, the two diastereotopic protons on C-3 were observed as two doublet of doublets at δ_H 4.17 (dd, J = 11.0 and 3.2) and δ_H 3.96 (dd, J = 11.0 and 5.5). H-2 was observed as a narrow multiplet at δ_H 3.34 and the two terminal epoxy protons were observed as a triplet at δ_H 2.89 (J = 4.5 Hz) and a doublet of doublet at δ_H 2.74 (J = 5.0, 2.6 Hz). The NMR data of this compound was in agreement with the data recorded in the literature (Terasaki et al., 2006). In LR-MS a peak was observed at *m/z* 359.3477 $[M+H_2O]^+$.

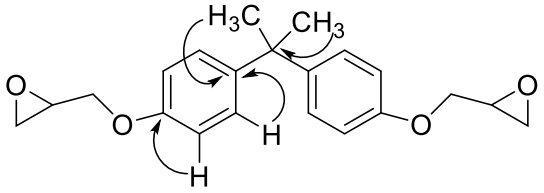


Figure 3.8: Key HMBC ($^1H \rightarrow ^{13}C$, arrow) and key $^1H-^1H$ COSY (bold) correlations for **3.17**.

Bisphenol A diglycidyl ether is an industrial chemical used as intermediate for the production of epoxyphenolic resins, polycarbonates, polyacrylates, plastics, and coatings for food / drink packaging (Saiyood et al., 2010). The area where the plant material was collected is often frequented by people and is also polluted with the food containers. The presence of **3.17** in the corms of *H. colchicifolia* corms is most likely as a result of pollution of the environment. It has been reported that **3.17** can be absorbed by plants (Saiyood et al., 2010).

3.3 ANTIDIABETIC ACTIVITY AND CYTOTOXICITY OF THE METHANOL EXTRACT OF *H. COLCHICIFOLIA*

3.3.1 Cytotoxicity screening

In type 2 diabetes, the body produces insulin, but the cell does not utilize it properly resulting in a high glucose concentration in the body. In the cell-based assay the effect of an extract or compound on the utilization of glucose is investigated to probe the antidiabetic activity of the extract or compounds. However, it was important to determine, whether the extract of *H. colchicifolia* was cytotoxic to the cell lines used.

The first assay determined the viability of liver and muscle cell lines in the presence of the crude extract of *H. colchicifolia*. The viability of a liver cell line (Chang) was determined by treating the cells with the extract at 12.5, 25 and 50 µg mL^{-1} and the viability was determined at 12, 24 and 48 h (Figure 3.9).

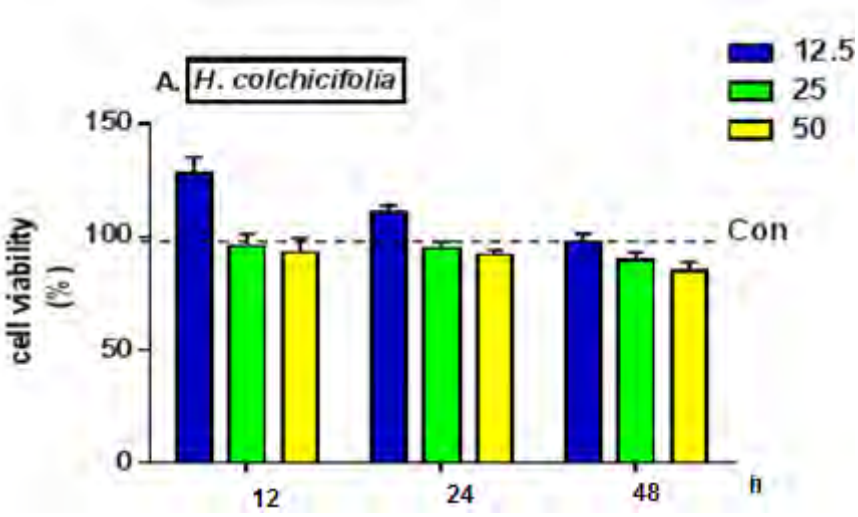


Figure 3.9: Cell viability of liver cell line (Chang) treated with *H. colchicifolia* extract at three concentrations (12.5, 25 and 50 µg mL^{-1}) after 12, 24 and 48 h. * = $p < 0.05$ in comparison to control.

The experiment was repeated with a muscle cell line (C2C12) and the results are shown in Figure 3.10.

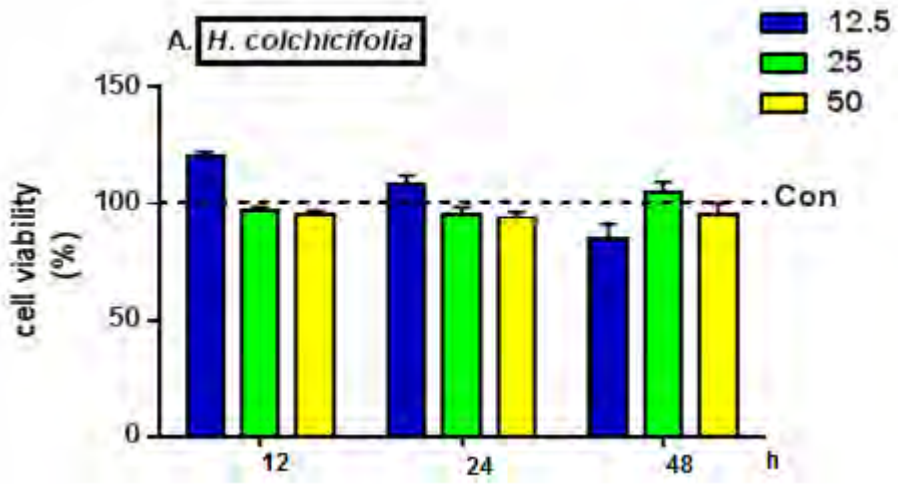


Figure 3.10: Cell viability of muscle cell line (C2C12) treated with *H. colchicifolia* extract at three concentrations (12.5, 25 and 50 µg mL^{-1}) after 12, 24 and 48 h. * = $p < 0.05$ in comparison to control.

The results of cell viability of both the liver cell line (Chang) and the muscle cell line (C2C12) when treated with different concentrations (12.5 - 50 µg mL^{-1}) of the MeOH extract of *H. colchicifolia* corms show that the cell viability remains constant after 48 h. It can be concluded that the MeOH extract (corms) of *H. colchicifolia* are not toxic to these cells.

3.3.2 Glucose utilization when the cells are treated with the methanol extract of *H. colchicifolia*

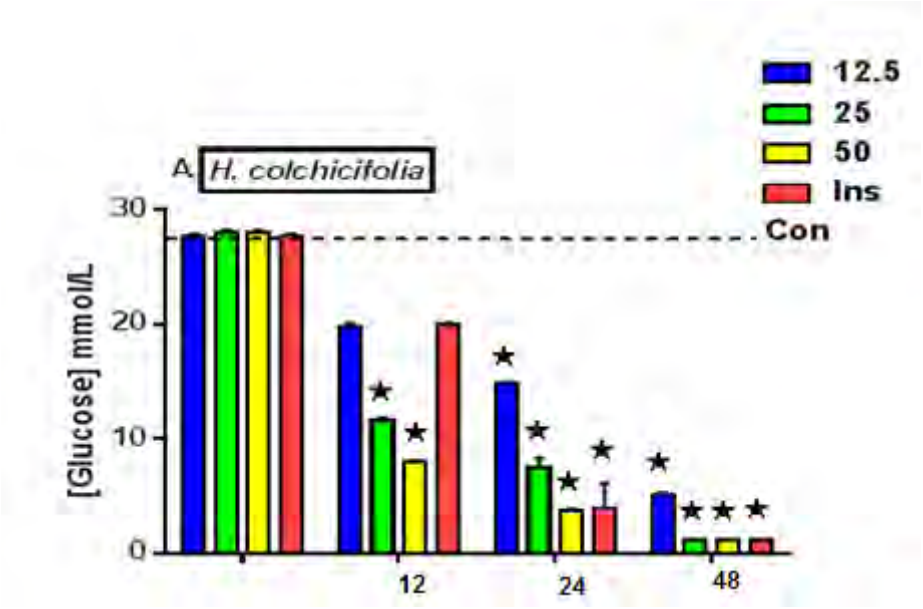


Figure 3.11: Effect of *H. colchicifolia* concentration (12.5, 25 and 50 µg mL^{-1}) on glucose utilization in a muscle cell line (C2C12) after 12, 24 and 48 h. * = $p < 0.05$ in comparison to control.

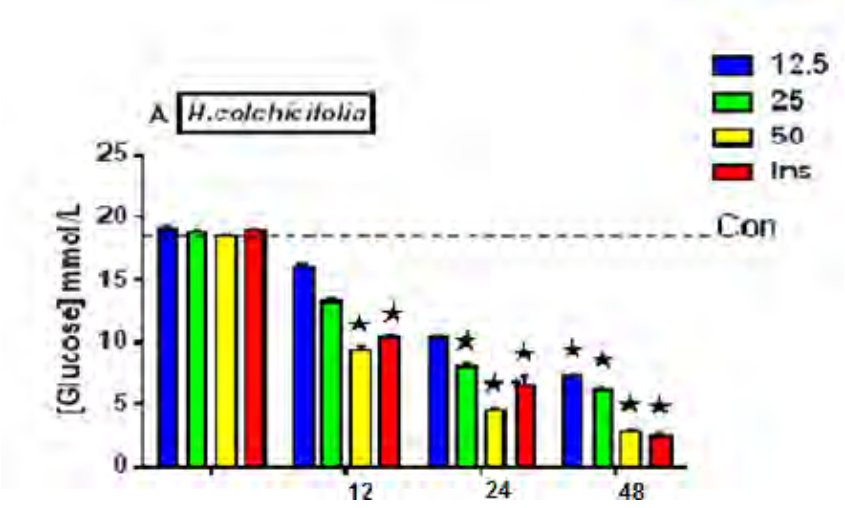


Figure 3.12: Effect of *H. colchicifolia* concentrations (12.5, 25 and 50 µg mL^{-1}) on glucose utilization in a liver cell line (Chang) after 12, 24 and 48 h. * = $p < 0.05$ in comparison to control.

The results showed that when the Chang liver cell line and the C2C12 muscle cell line were treated with the MeOH extract of *H. colchicifolia*, the level of glucose significantly decreased after 12 h at extract concentrations of 25 and 50 µg mL^{-1}, whereas for an extract concentration of 12.5 µg mL^{-1}, the level of glucose significantly decreased only after 24 h.

The results further showed that the MeOH extract of *H. colchicifolia* had glucose lowering effects and low toxicity *in vitro*. An unpublished study in rats where diabetes was induced by STZ, reported that the methanol extract of corms of *H. colchicifolia* and *H. hemerocallidea* reduced the level of blood glucose in 5 h at 45 mg kg^{-1} (Thring and Weitz, 2006). Ojewole (2006) also observed that the aqueous extract of the related species *H. hemerocallidea* (800 mg kg^{-1}) reduced blood glucose in experimental animals after 8 h of treatment.

3.3.3 Glucose utilization when the cells were treated with the compounds

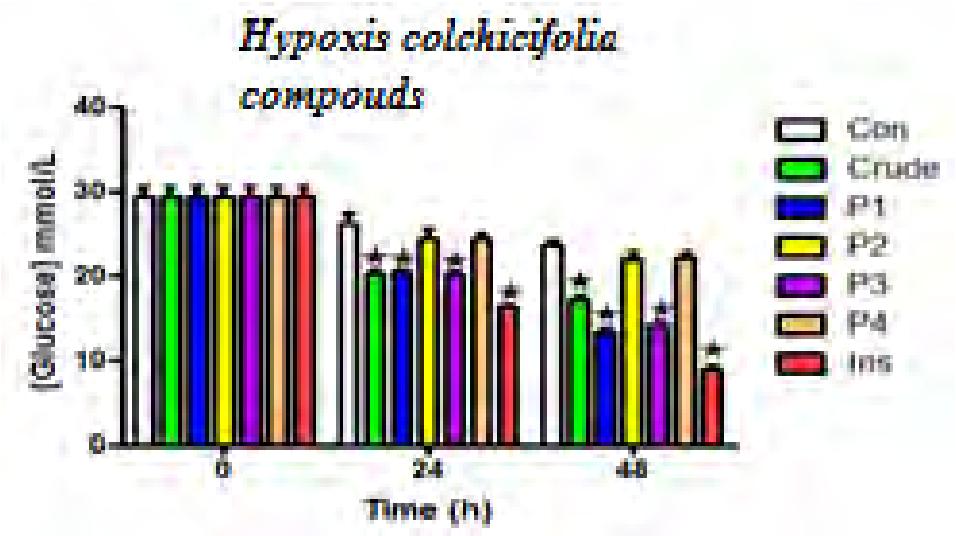


Fig 3.13: The effects of *H. colchicifolia* compounds on glucose utilization in muscle (C2C12) cell line. * = $p < 0.05$ in comparison to the control.

When the muscle cell line was treated with the individual fractions from the MeOH extract of *H. colchicifolia* [P1 (mixture of sugars and tannins), P2 (**3.1**) P3 (**3.14** and **3.15**) and P4 (**3.16**)], the most active fractions were P1 and P3.

This study was the first to prove antidiabetic activity of the MeOH extract of *H. colchicifolia*. It can be concluded that *H. colchicifolia* has potential for future antidiabetic drug development.

3.4 EXPERIMENTAL

3.4.1 General experimental procedures

The solvents used in this project, i.e. ethyl acetate, DCM, hexanes (mixture of hexane isomers), and MeOH were of analytical grade. HPLC grade MeOH was used for the HPLC procedures. The water was purified in a PureLab Ultra ELGA® system.

For the separation and purification of compounds, centrifugal chromatography (Chromatotron model 7924, Harrison Research) or column chromatography were used. The circular

Chromatotron plates were coated with preparative silica gel (2 or 4 mm thickness, Merck 7749 with gypsum binding agent). Column chromatography was performed with silica gel 60 F_{254}, (40-63 μm, Merck). Thin-layer chromatography (TLC) plates (Kieselgel 60 F_{254}, 0.25 mm) stained with anisaldehyde/sulfuric acid were used for the qualitative analysis of fractions and compounds. The stain solution was made as follows: In a 1 L volumetric flask, 845 mL of MeOH was cooled in an ice bath, while shaking to ensure homogeneity, 100 mL of acetic acid was added followed by addition of 40 mL of concentrated sulfuric acid and 5 mL of anisaldehyde. The solution was stored in a fridge.

HPLC was used for the isolation and qualitative analysis of the compounds. The isolation was performed by semi-preparative HPLC using a LC-20AB P2000 pump equipped with a SIL-20A autosampler and a SPD-20A UV detector (all supplied by Shimadzu). The isolation was achieved on a Luna 5μm C18 (250 x 10 mm) semi-preparative column (Phenomenex). A mobile phase consisting of 1% acetic acid in water (solvent A) and MeOH (solvent B) in a gradient program [45% MeOH (3 min), 45 – 50% MeOH (5 min), 50 - 55% MeOH (9.5 min), 55% (0.5 min)] at a flow rate of 3 mL min^{-1}. Samples (15 mg mL^{-1}) were injected (120 μL per injection) onto the column, which was maintained at a temperature of 40 ºC, and the eluant was monitored at wavelength range of 210 - 450 nm with a diode array UV detector.

Qualitative analyses of fractions were carried out on a Kinetex 2.6 μm C18 (2) (100 x 4.6 mm) column (Phenomenex). The separation was achieved using the same mobile phase described for the isolation of the compounds but a linear gradient of 15% in 5 minutes (40 to 90%) at a flow rate of 0.4 μL min^{-1}, using a detection wavelength of (210 - 450 nm).

NMR spectra (^{1}H, ^{13}C, COSY, HSQC, HMBC and DEPT) Bruker Avance III 500 or Bruker Avance III 400 spectrometers were used. All spectra were recorded in deuterated solvents ($CDCl_3$ and CD_3OD) at 30 °C using either a 5 mm BBOZ probe or a 5 mm TBIZ probe. Chemical shifts (δ) are given in parts per million (ppm) and are referenced to a residual protonated solvent peak ($CDCl_3$: ^{1}H 7.26, ^{13}C, 77.0, methanol-d_4: ^{1}H 3.31, ^{13}C, 49.1). Peak multiplicities are designated as s for singlet, d for doublet, dd for doublet of doublet t for triplet, dt for doublet of triplet, m for multiplet. Coupling constants (*J*) are given in Hz.

High-resolution mass spectral data were acquired on a time-of-flight Waters LCT Premier mass spectrometer using electrospray ionization in the positive or negative mode. Infrared spectra were recorded on a Perkin Elmer FT-IR (Fourier transform infrared spectroscopy)

spectrometer with universal ATR sampling accessory, optical rotations were determined using an ADP 440+ model polarimeter manufactured by Bellingham and Stanley.

3.4.2 Collection of plant material

The fresh corms of *H. colchicifolia* were collected in Hayfields, Pietermaritzburg in KwaZulu-Natal. The plant was identified by Prof. Drewes and a voucher (van Heerden 8) was deposited in the Bews Herbarium of the School of the Life Science, Pietermaritzburg campus, University of KwaZulu-Natal. The corms were washed with water and then cut into small pieces. It was dried in an oven at 30 ºC for 3 days until reaching a constant weight. It was then ground with a mechanical mill to afford a mass of 423 g.

3.4.3 Extraction and isolation of hypoxoside derivatives

The powdered corms (423 g) were extracted successively with DCM:MeOH (1:1) and MeOH, for 24 h at room temperature. After each extraction, the solvent was filtered using Whatman® No 1 filter paper and concentrated under vacuum using a rotary evaporator. The extracts were then dried under high vacuum. The yields were 13.6 g for the MeOH:DCM (1:1) extract and 25.2 g for the MeOH extract. On TLC and HPLC the contents of these two extracts were very similar and further investigations were performed on the methanol extract only.

A portion of the dried MeOH extract (1 g) was fractionated by semipreparative HPLC. The extract was weighed out accurately and dissolved in HPLC-grade MeOH to obtain a concentration of 15 mg mL^{-1}. The fresh solution (120 µL) was injected onto a semi-preparative HPLC column with chromatography conditions described previously. Four fractions, F1 (R_t 4.291), F2 (R_t 17.024), F3 (R_t 17.677) and F4 (R_t 18.335), were collected manually according to the chromatographic profile shown in Figure 3.14. After repeated injections, fractions with the same retention times were combined and concentrated with a rotary evaporator under vacuum until complete removal of water. The yields were 55.6 mg, 34.4 mg, 19.7 mg and 23.1 mg for fractions F1 to F4, respectively.

The samples were dried under high vacuum to yield the compounds as brown powders.

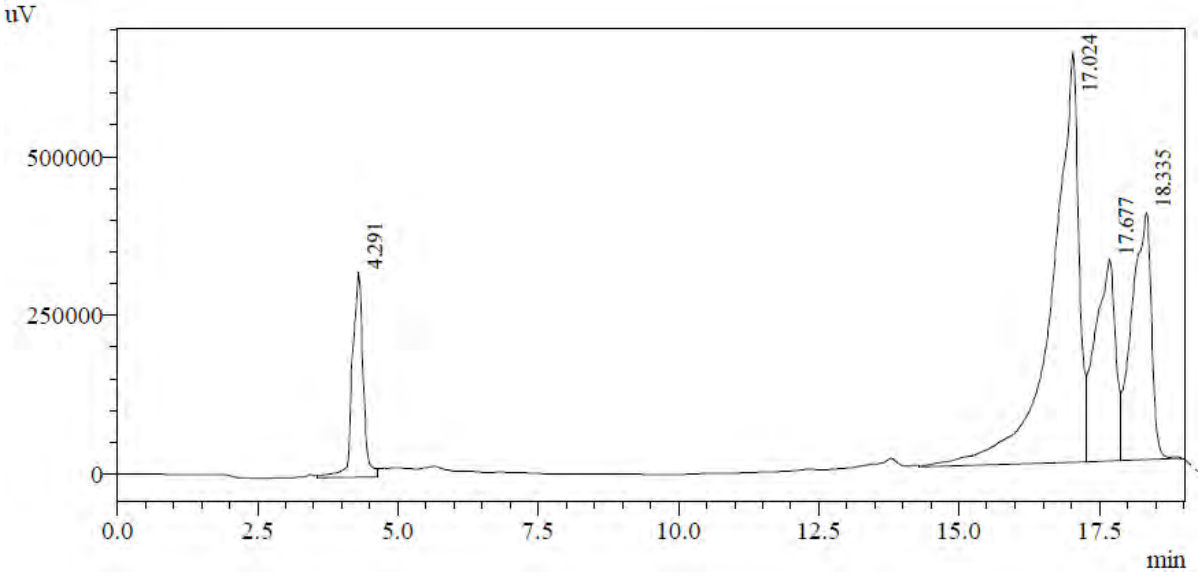


Figure 3.14: HPLC profile of the methanol extract of *H. colchicifolia* on the preparative column.

Solutions with a concentration of 1 mg mL^{-1} in MeOH were prepared of each fraction separately were injected in an analytical column with the aim to test the purity. Under the same chromatographic conditions described anteriorly were observed purity of each fraction as described in the Figures 3.15 to 3.18.

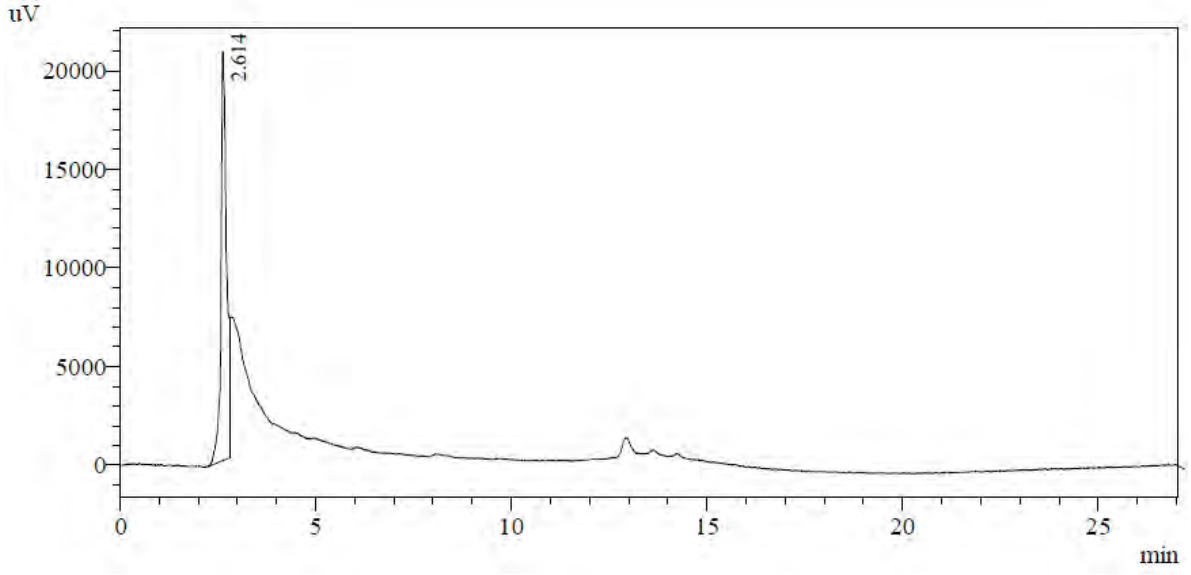


Figure 3.15: HPLC profile of the fraction F1 on analytical column.

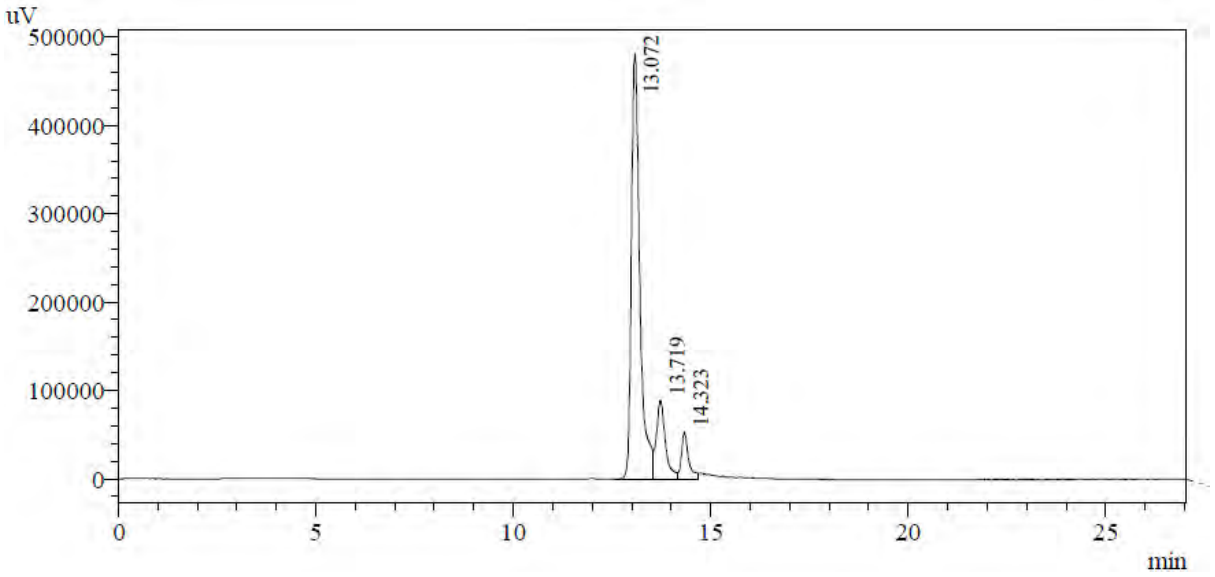


Figure 3.16: HPLC profile of the fraction F2 in analytical column

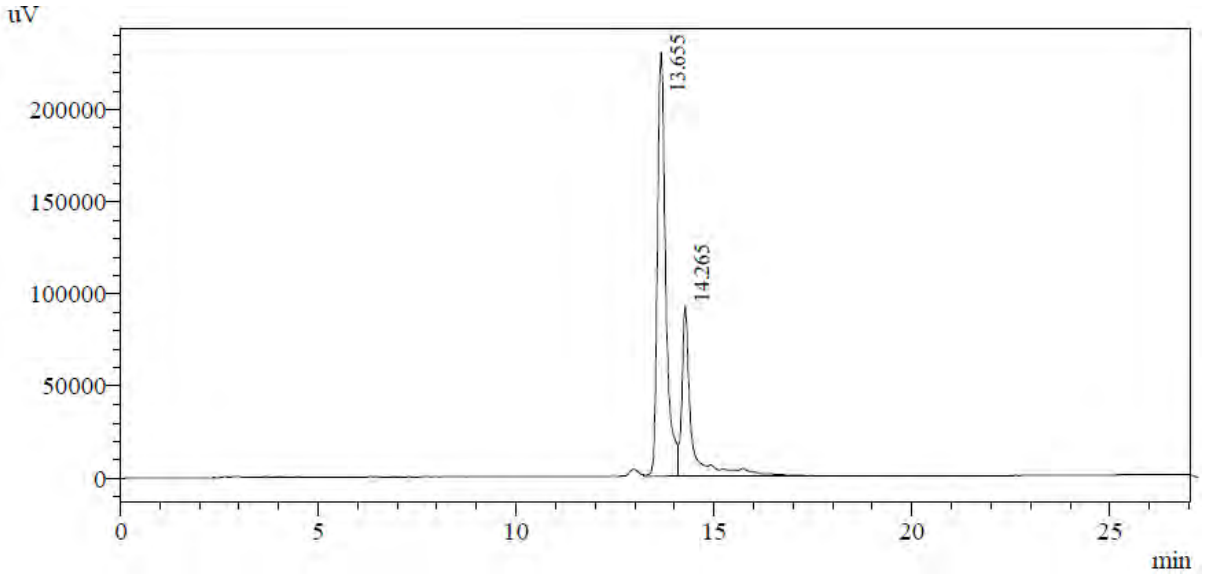


Figure 3.17: HPLC profile of fraction F3 in analytical column.

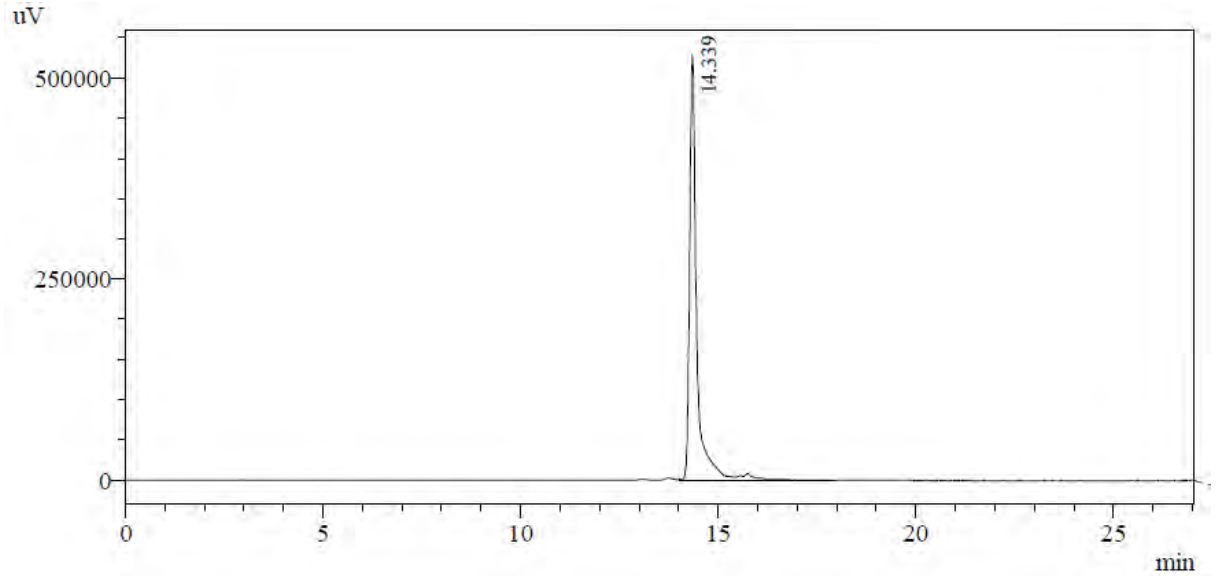


Figure 3.18: HPLC profile of fraction F4 in analytical column.

After analysing the NMR data, it was concluded that the fraction F1 corresponded to a complex mixture of tannins and sugars. This fraction was not investigated further. F2 corresponded to hypoxoside (**3.1**), F3 contained a mixture of dehydroxyhypoxoside A (**3.14**) and dehydroxyhypoxoside B (**3.15**) and F4 consisted of bis-dehydroxyhypoxoside (**3.16**).

Hypoxoside (**3.1**) was isolated as a brown solid. ^{1}H NMR (500 MHz, CD_3OD) δ_H 7.12 (2H, d, *J* = 8.3 Hz, H-5',5''), 6.92 (1H, d, *J* = 2.0 Hz, H-2'), 6.90 (1H, d, *J* = 2.0 Hz, H-2''), 6.87 (1H, dd, *J* = 8.3, 2.0 Hz, H-6''), 6.83 (1H, dd, *J* = 8.3, 2.0 Hz, H-6'), 6.57 (1H, d, *J* = 15.7 Hz, H-1), 6.13 (1H, dt, *J* = 15.7, 5.7 Hz, H-2), 4.79 (1H, d, *J* = 7.5 Hz, H-1''' or H-1''''), 4.75 (1H, d, *J* = 7.5 Hz, H-1''' or H-1''''), 3.90 (2H, d, *J* = 12.2, H_a-6''',6''''), 3.72 (2H, br d, *J* = 12.2, H_b-6''',6''''), 3.52-3.44 (4H, m, H-2''',2''',3''',3''''), 3.50-3.41(1H, m, H-4''',4''',5''',5''''), 3.28 (2H, dd, *J* = 7.0 and 1.5 Hz, H-3) (Plate 1a); ^{13}C NMR (100 MHz, CD_3OD) δ_C 148.5, 148.1 (C-3',3''), 146.9, 146.3 (C-4',4''), 134.6 (C-1'), 131.8 (C-1), 124.7 (C-6''), 124.5 (C-2), 120.3 (C-1''), 120.1 (C-2''), 119.4 (C-6'), 118.9, 118.5 (C-5',5''), 114.5 (C-2'), 104.4, 104.0 (C-1''',1''''), 86.3 (C-4), 83.3 (C-5), 78.4, 78.3 (C-5''',5''''), 77.64, 77.60 (C-3''',3''''), 74.9, 74.8 (C-2''',2''''); 71.3 (2C, C-4''',4'''') 62.4 (2C, C-6''',6''''), 23.3 (C-3) (Plate 1b); $[\alpha]_D^{20}$ = -70.1^{o}; HRMS *m/z* 605.1868 $[M + H]^+$ $C_{29}H_{33}O_{14}$ (cald 605.1870 for 100%). UV (MeOH) λ_{max} (nm) 210, 257, 297. IR ν_{max} (cm^{-1}) OH (3332); CH (2893).

Dehydroxyhypoxoside A (**3.14**) was isolated as a brown solid substance. ^{1}H NMR (500 MHz, CD_3OD): δ_H 7.33 (2H, d, *J* = 8.8 Hz, H-2',6'), 7.13 (1H, d, *J* = 8.2 Hz, H-5''), 7.04 (2H, d, *J* = 8.8 Hz, H-3',5'), 6.90 (1H, d, *J* = 2.1 and 2.0 Hz, H-2''), 6.87 (1H, d, H-6''); 6.58 (1H, d, *J* = 17.1 Hz, H-1), 6.15 (1H, m, H-2), 4.91 (1H, d, *J* = 8.0 Hz, H-1'''), 4.90 (1H, d, *J* = 7.8 Hz, H-1'''), 4.78 (1H, d, *J* = 7.5 Hz, H-1''' or H-1''''), 4.75 (1H, d, *J* = 7.5 Hz, H-1''' or H-1''''), 3.89 (2H, br d, *J* = 12.0 Hz, H_a-6'''), 3.75-3.67 (1H, m, H_b-6'''), 3.52-3.36 (4H, m, H-2''',2'''',3''', 3''''), 3.50-3.41 (4H, m, 4''',4'''',5''',5''''), 3.32 (2H, d, *J* = 6.0 Hz, H-3), (Plate 2a); ^{13}C NMR (100 MHz, CD_3OD): δ_C 158.5 (C-4'), 148.5 (C-4''), 147.06 or 146.7 (C-3''), 133.1 (C-1'), 131.8 (C-1), 128.9 (C-3'), 124.7 (C-6''), 124.1 (C-2), 120.3 (C-1''), 120.1 (C-2''), 118.9 or 118.5 (C-5''), 117.9, 117.7 (2C, C-3',5'), 104.4 or 104.0 (C-1''',1''''), 101.0, 100.7 (2C, C-1''', 1''''), 86.3 (C-4), 83.1 (C-5), 78.4, 78.3 (2C, C-5''',5'''') or 78.21, 78.18 (2C, C-5''',5''''), 78.02, 78.01 (2C, C-3''',3''''), or 77.64, 77.60 (2C, C-3''',3''''), 74.9, 74.8 (2C, C-2''',2'''') or 74.93, 71.91 (2C, C-2''',2''''), 71.3 or 69.9 (2C, C-4''',4''''), 62.55, 62.51, 62.4, (2C, C-6''',6''''), 23.3 (C-3) (Plate 2c); $[\alpha]^{20}$ = -67.3 HRMS *m/z* 589.1915 $[M - H]^+$ $C_{29}H_{33}O_{13}$ (cald 589.1921 for 100%); UV (MeOH) λ_{max} (nm) 210, 257, 297; IR ν_{max} (cm^{-1}) OH (3332), CH (2893.2).

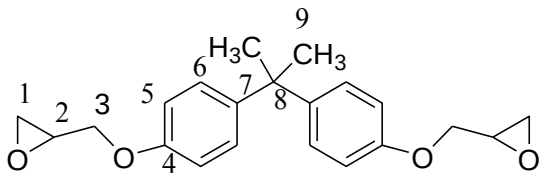
Dehydroxyhypoxoside B (3.15) was isolated as a brown solid substance. ^{1}H NMR (500 MHz, CD_3OD): δ_H 7.35 (1H, t, *J* = 8.8 Hz, H-2'',6''), 7.13 (1H, d, *J* = 8.2 Hz, H-5'), 7.04 (2H, d, *J* = 8.9 Hz, H-3'',5''), 6.92 (1H, d *J* = 2.0, H-2'), 6.83 (1H, dd, *J* = 8.4 Hz and 1.9 Hz, H-6'), 6.58 (1H, d, *J* =17.1 Hz, H-1), 6.15 (1H, m, H-2), 4.78 (1H, d, *J* = 7.5 Hz, H-1'''), 4.91 (1H, d, *J* = 8.0 Hz, H-1'''), 4.90 (1H, d, *J* = 7.8 Hz, H-1'''), 4.78 (1H, d, *J* = 7.5 Hz, H-1''' or H-1''''), 4.75 (1H, d, *J* = 7.5 Hz, H-1''' or H-1''''), 3.89 (2H, br d, *J* = 12.0 Hz, H_a-6'''), 3.75-3.67 (1H, m, H_b-6'''), 3.52-3.36 (4H, m, H-2''',2'''',3''', 3''''), 3.50-3.41 (1H, m, 4''',4'''',5''',5''''), 3.32 (2H, d, *J* = 6.0 Hz, H-3), (Plate 2a). ^{13}C NMR (100 MHz, CD_3OD): δ_C 158.7 (C-4''), 148.5 (C-4''), 147.06-146,7 (C-3'), 133.8 (2C, C-2'',6''), 134.5 (C-1''), 131.7 (C-1), 124.1 (C-2), 120.1 (C-2'), 119.4 (C-6'), 114.5 (C-2'), 104.4 or 104.0 (C-1''',1''''), 101.0, 100.7 (2C, C-1''', 1''''), 86.3 (C-4), 83.1 (C-5), 78.4, 78.3 (2C, C-5''',5'''') or 78.21, 78.18 (2C, C-5''',5''''), 78.02, 78.01 (2C, C-3''',3''''), or 77.64, 77.60 (2C, C-3''',3''''), 74.9, 74.8 (2C, C-2''',2'''') or 74.93, 71.91 (2C, C-2''',2''''), 71.3 or 69.9 (2C, C-4''',4''''), 62.55, 62.51, 62.4, (2C, C-6''',6''''), 23.3 (C-3) (Plate 2b); $[\alpha]^{20}$ = -67.3 HRMS *m/z* 589.1915 $[M - H]^+$ $C_{29}H_{33}O_{13}$ (cald. 589.1921); UV (MeOH) λ_{max} (nm) 210, 257, 297; IR ν_{max} (cm^{-1}) OH (3332), CH (2893.2).

Bis-dehydroxyhypoxoside (**3.16**) was isolated as a brown, amorphous solid. ^{1}H NMR (500 MHz, CD_3OD): δ_H 7.35 (2H, d, *J* = 9.0, H-2",6"), 7.33 (2H, d, *J* = 9.0, H-2',6'), 7.04 (4H, d, *J* = 9 Hz, H-3',3", 5',5"), 6.64 (1H, br d, *J* = 15.8 Hz, H-1), 6.17 (1H, dt, *J* = 15.8, 5.8 Hz, H-2), 4.91 (1H, d, *J* = 8.0 Hz, H-1'",1""), 4.90 (1H, d, *J* = 7.8 Hz, H-1'",1""), 3.89 (2H, dd, *J* = 12.0, 2.2 Hz, H_a-6'",6""), 3.71 (1H, dd, *J* = 12.2, 1.3 Hz, H_b-6'" or 6""), 3.70 (1H, dd, *J* = 12.2, 1.3 Hz, H_b-6'" or 6""), 3.52-3.44 (4H, m, H-2'",2"",3'",3""), 3.43-3.88 (1H, m, H-4'",4"",5'",5""), 3.28 (2H, dd, *J* = 7.0 and 1.5 Hz, H-3) (Plate 3a). ^{13}C NMR (100 MHz, CD_3OD): δ_C 158.7 (C-4") 158.5 (C-4'), 133.8 (2C, C-2",6"), 133.1 (C-1'), 131.7 (C-1), 128.9 (2C, C-2',6'), 124.1 (C-2), 119.7 (C-1"), 117.9, 117.7 (4C, C-3',3",5',5"), 101.0, 100.7 (2C, C-1'", 1""), 86.5 (C-4), 83.2 (C-5), 78.21, 78.18 (2C, C-5'",5""), 78.02, 78.01 (2C, C-3'",3""), 74.93, 71.41 (2C, C-2'",2"") 71.41, 71.37 (2C, C-4'",4""); 69.9 (C-5'"); 62.55, 62.51 (C-6'",6""), 23.4 (C-3) (Plate 3c); $[\alpha]_D^{20}$ = -64.4^{o}; HRMS *m/z* 597.1950 $[M + Na]^+$ $C_{29}H_{34}O_{12}Na$ (cald 597.1948); UV (MeOH) λ_{max} (nm) 210, 259; IR ν_{max} (cm^{-1}) OH (3332), CH (2893).

3.4.4 Extraction and isolation of bisphenol A diglycidyl ether (3.17)

Oven–dried (30 °C) and ground corms of *H. colchicifolia* (246 g) were extracted successively with DCM and MeOH at room temperature for 24 h to afford 5.1 g of the MeOH extract and 326 mg of the DCM extract.

The methanol extract (2.5 g) was fractionated on a short silica gel column by elution with increasing polarity of hexanes:EtOAc to yield 4 fractions after combination according to TLC profile, the 4th being a MeOH wash. Fraction 3 (corresponding to pure EtOAc) (236 mg) was purified twice on a Chromatotron with EtOAc:hexanes (3:7) resulting in the isolation of bisphenol A diglycidyl ether (**3.17**, 2 mg).



3.17 Bisphonol A diglycidyl ether

Bisphenol A diglycidyl ether (**3.17**) was isolated as yellow, amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.13 (4H, d, *J* = 9.0 Hz, H-6), 6.81 (4H, d, *J*= 9.0 Hz, H-5), 4.17 (2H, dd, *J*= 11.0, 3.2 Hz, H-3a), 3.96 (2H, dd *J* = 11.0, 5.5 Hz, H-3b), 3.34 (m, 2H, H-2), 2.89 (2H, t, *J*

= 4.5 Hz, H-1a), 2.74 (2H, dd, *J* = 5.0, 2.6 Hz, H-1b), 1.64 (6H, s, H-9) (Plate 4a). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 157.4 (C-4), 143.3 (C-7), 127.8 (C-6), 114.3 (C-5), 68.5 (C-3), 50.0 (C-2), 44.6 (C-1), 41.7 (C-8), 32.0 (C-9) (Plate 4c); LR-MS *m/z* 359.3477 $[M + H_2O]^+$, $C_{21}H_{24}O_4 + H_2O$, (calcd. 359.1814).

3.4.5 EVALUATION OF THE EFFECTS OF PLANT EXTRACTS AND COMPOUNDS ON GLUCOSE METABOLISM IN LIVER AND SKELETAL MUSCLE CELLS

Cell culture experiments were performed by Ntethelelo Sibiya in the Physiology Research group of the School of Laboratory Medicine and Medical Science, University of KwaZulu-Natal.

3.4.5.1 Experimental cells

The cell lines used for the study were muscle (C2C12) and liver (Chang) cells which were donated by the Medical Research Council, Cape Town, South Africa. These cells were cultured in 25 mL flasks in DMEM and EMEM, respectively. Cells were used within 10 passage numbers to limit batch-to-batch variation. Culture media were supplemented with 10% foetal calf serum (FCS), 1% L-glutamate and 1% penicillin/ streptomycin/ fungizone.

3.4.5.2 Cell culture protocol

Cell culture was conducted using a well-established cell culture protocol. Briefly, muscle and liver cells were cultured in 10 mL DMEM or EMEM, respectively, at 37 ºC, 5% CO_2 and 89% relative humidity. The cells were allowed to grow and become confluent; thereafter the confluent cells were trypsinized with trypsin (1 mL) after washing three times with PBS. The trypsinized cells were sub-cultured into new flasks and some were stored in 10% DMSO at -80 ºC for subsequent studies.

3.4.5.3 Cell seeding

Both muscle (C2C12) and liver (Chang) cells were seeded at a density of 1.5×10^5 cells/mL in a 24-well cell culture plate. Both cell lines were allowed to grow and reach 80% confluence monolayer.

3.4.5.4 Experimental design

The effect of plant extract and compounds, were evaluated on glucose utilization and glycogen content in muscle and liver cells.

3.4.5.5 Experimental protocol

Each cell line was divided as follows: group 1 was treated with media containing glucose only, which served as the control, group 2 was treated with standard drug (insulin at 0.04 mg mL^{-1}), and group 3 was treated with plant extract at various doses (12.5, 25 and 50 µg mL^{-1}). This experimental protocol was used for all plant extracts and isolated compounds. To search for the influence of plant extracts on insulin action, all plant extracts or isolated compounds were combined with insulin.

3.4.2.5.6 Glucose utilization model

The glucose utilization experiment was performed as previously described by van de Venter et al. (2008) with slight modifications. DMEM and EMEM contained glucose at a concentration of 28 mM. After seeded cells reached 80% confluence, the old media was replaced with fresh media. Plant extracts (100 µL) and plant extracts plus insulin (100 µL) was added into the media (1 mL) for treatment in each well. The culture plate was then incubated at the aforementioned conditions. Glucose concentrations were measured at 0, 12, 24 and 48 h intervals using a glucometer.

3.4.5.7 Cell viability assay

Cell proliferation was measured using the CellTiter-Glo Luminescent Cell Viability Assay (Technical Bulletin, Promega, Madison). Cells were seeded in 96-well plates at a density of 10 000 cells/well and incubated for 12, 24 and 48 h with the plant extracts (12.5, 25 and 50 µg mL^{-1}). After every 12, 24 and 48 h, plates were equilibrated at room temperature for 30

minutes, assay reagent (100 µL) was added to each well, thereafter the plate was placed on an orbital shaker for 2 minutes to induce cell lysis. After shaking, plates were incubated at room temperature for 10 minutes to stabilize the luminescence resonance, thereafter the luminescence was read on a Promega Microplate Luminometer. All plates had control wells containing media without cells for background correction. Data were expressed as percentage of viable cells (i.e. treatment value-blank/control value-blank), mean ± SEM for three replicates.

3.4.5.8 Statistical analysis

All data is expressed as means ± standard error of means (SEM). Statistical analysis was done using GraphPad Prism Instat Software (version 5.00, GraphPad Software, San Diego, California, USA). One-way analysis of variance (ANOVA) followed by Tukey-Kramer was used for analysis of differences between control and experimental groups. Values of $p < 0.05$ were taken to imply statistical significance.

CHAPTER 4: A PHYTOCHEMICAL AND ANTIDIABETIC INVESTIGATION OF *TERMINALIA SERICEA*

4.1 INTRODUCTION AND LITERATURE REVIEW

4.1.1 The family Combretaceae

The Combretaceae (Figure 4.1) is a family of flowering plants belonging to the order Myrtales, with about 600 species of trees, shrubs and lianas in 18 genera (Eloff et al., 2008). Combretaceae are common in subtropical and tropical regions (Fyhrquist et al., 2006).

Figure 4.1: An example of a flower of Combretaceae.
http://en.wikipedia.org/wiki/Combretaceae

In traditional medicine in Africa several species of the Combretaceae are used to treat many diseases, such as pneumonia, fever, headaches, abdominal disorders, venereal diseases including syphilis, earache, gallstones, diarrhoea, dysentery, gastric ulcers, bilharziasis, hookworm, nosebleeds, sore throats, colds, chest coughs, conjunctivitis, dysmenorrhoea, infertility in women, fattening babies, leprosy, scorpion and snake bites, among others (Eloff et al., 2008).

4.1.2 The genus *Terminalia*

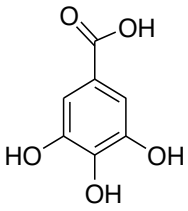
Terminalia L. (Combretaceae) is a genus of large trees and includes 100 species disseminated in the tropical areas of the world. It is estimated that 40 species of *Terminalia* are endemic to Africa, 11 of which occur in the southern African region (Eloff et al., 2008). The name of this genus is derived from the Latin 'terminus', referring to the fact that the leaves appear at the very tips of the shoots. Trees of this genus are well known especially as a potential source of secondary metabolites, e.g. flavonoids, triterpenes and their derivatives, tannins, among others. Some of these compounds have antifungal, antibacterial, anti-cancer and hepatoprotective effects.

4.1.3 Uses

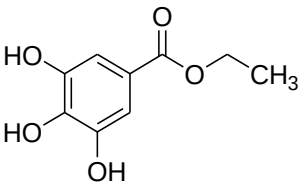
Terminalia species are used for diverse therapeutic applications such as diabetes, female infertility, abdominal pain, diarrhoea, and vermifuge, and known as a rich source of secondary metabolites, such as pentacyclic triterpenes and their glycoside derivatives, flavanones and chalcones (Ponou et al., 2011).

Andrade-Cetto and Heinrich reported that two plants of the Combretaceae, *Terminalia catappa* L. (fruit) and *Combretum farinosum* Kunth (raw sap) are used in Mexico to treat diabetes (Andrade-Cetto and Heinrich, 2005). Kumar et al. found antidiabetic activity for the ethanol extract of *T. chebula* Retz. (fruits) *in vivo* (Kumar et al., 2006).

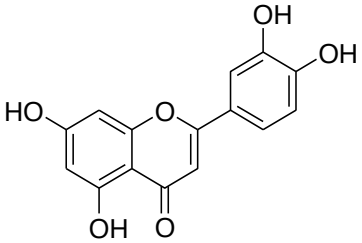
Phenolic compounds such as gallic acid (**4.1**), ethyl gallate (**4.2**) and luteolin (**4.3**) with antineoplastic activities against human cancer cell lines have been isolated from the stem bark and leaves of *T. arjuna* (Roxb. Ex DC.) Wight & Am. (Fyhrquist et al., 2006), was also found that gallic acid is responsible for the antifungal activity of *T. nigrovenulosa* Pierre (bark) (Nguyen et al., 2013). *T. chebula* (fruit) have been found to contain the tannin chebulinic acid (**4.4**), which decreases cell viability and inhibits cell proliferation in MCF 7 breast cancer, PC 3 prostate cancer and HOS osteosarcoma cell lines in a dose-dependent manner (Fyhrquist et al., 2006).



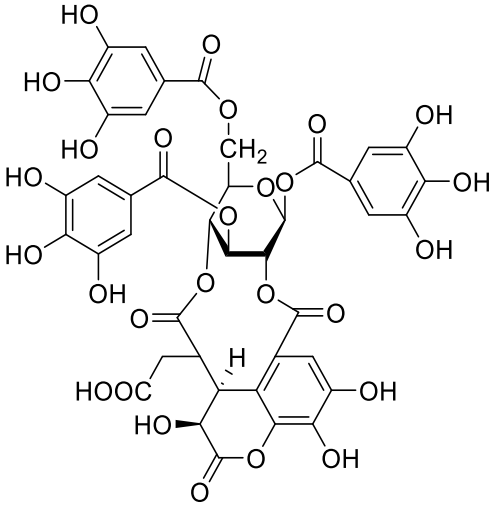
4.1 Galic acid



4.2 Ethyl gallate

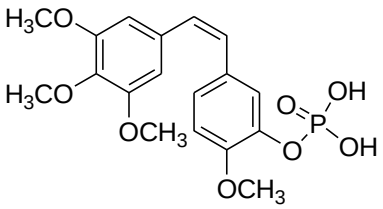


4.3 Luteolin



4.4 Chebulinic acid

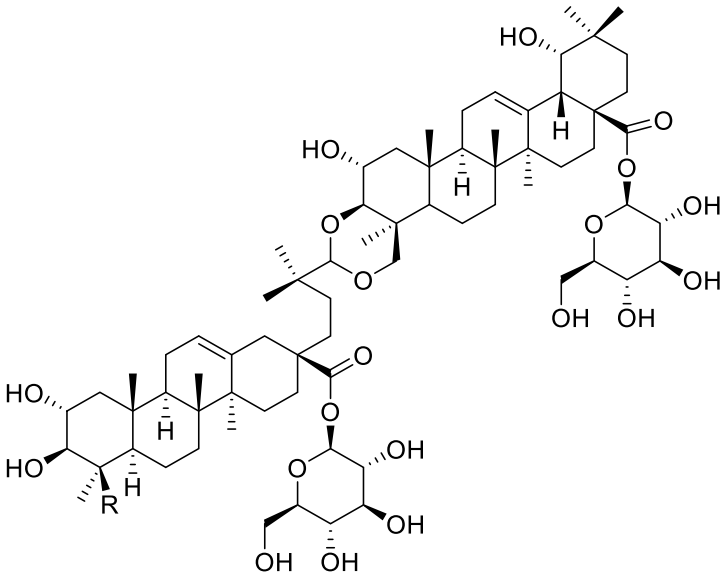
The combretastatins are a group of stilbenes isolated from the stem bark of the South African bush willow tree, *Combretum caffrum* (Eckl.& Zegh.) Kuntze. A synthetic compound, combretastatin A-4-P (**4.5**), is a potential anticancer agent that is currently in clinical trial for the treatment of cancer (Cancer_Research_United_Kindom, 2014).



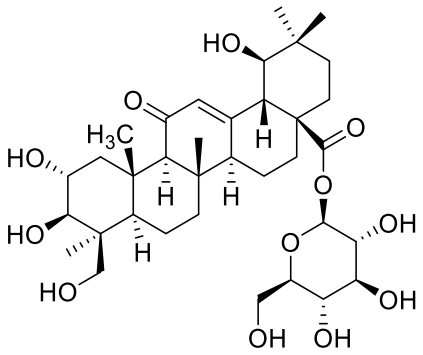
4.5 Combretastatin A-4 phosphate

The ethanol extract (stems) of *T. phanerophlebia* Engl. & Diels have anti-inflammatory activity (Nair et al., 2012). From the bark of *T. ivorensis* A. Chev., three saponins, including two dimeric triterpenoid glucosides possessing an unusual skeleton, ivorenoside A (**4.6**) and ivorenoside B (**4.7**), and ivorenoside C (**4.8**), were isolated. Compounds **4.7** and **4.8** exhibited scavenging activity against DPPH and $ABTS^{+}$ radicals with IC_{50} values comparable to that of Trolox® (**4.9**) and **4.6** showed antiproliferative activity against MDA-MB-231 breast cancer

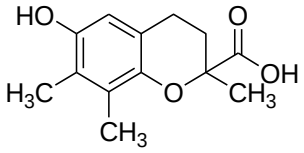
and HCT11 human cancer cell lines with IC_{50} values of 3.96 and 3.43 μM, respectively (Ponou et al., 2010).



4.6 R=CH_2OH: Ivorenoside A
4.7 R=COOH: Ivorenoside B



4.8 Ivorenoside C



4.9 Trolox®

The extracts of the leaves, stem and roots of *T. mollis* have antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Bacillus anthracis* and antifungal activity against *Candida albicans* (Moshi et al., 2006).

4.1.4 Review of *Terminalia sericea*

Terminalia sericea Burch. ex. DC (Figure 4.2) is a medium-sized deciduous tree widely distributed in the tropical and warm temperate regions, especially in Africa (Moshi and Mbwambo, 2005). The plant has wide-spread traditional uses in eastern and southern African countries (Moshi and Mbwambo, 2005).

Figure 4.2: A young tree of *Terminalia sericea.*
www.nparks.gov.sg/nursery/uploadfiles/terminaliasericea

T. sericea has reddish-brown branches and the leaves are crowded at the ends of branches. The leaves are narrowly obovate-elliptic with smooth margins, blue-green above, paler below and densely covered with silvery hairs. It flowers mostly in September - January. The flowers are in axillary spikes and are pale yellow to creamy white. The fruit is an oval nut surrounded by a flat wing. The fruits are often parasitized and grow deformed (Masupa, 2012). *T. sericea* occurs in a variety of types of open woodlands particularly on sandy soils. It grows from Tanzania and the Democratic Republic of the Congo southwards to Angola and Namibia, Zimbabwe, Botswana, South Africa and Mozambique (Masupa, 2012).

T. sericea is an important plant in traditional medicine and is in the top 50 most important African medicinal plants (Eloff et al., 2008; Masoko et al., 2005). The aqueous extracts of the leaves and roots and infusions are used in traditional medicine to treat coughs, diarrhoea and stomach aches and the leaves can be used as an antibiotic for wounds. The wood has commercial value and is used for constructing huts and for solid structures.

The extract of *T. sericea* (roots) inhibited the enzyme α-glucosidase (IC_{50} = 92 μg mL^{-1}) better than acarbose (IC_{50} = 131 μg mL^{-1}) and inhibited HIV reverse transcriptase (IC_{50} = 43 μg mL^{-1}) more effectively than Adriamycin® (IC_{50} 100 μg mL^{-1}) (Tshikalange et al., 2008). It was also reported that the acetone extract of *T. sericea* (bark) were active against multidrug-resistant *Mycobacterium tuberculosis* with a MIC less than 100 μg mL^{-1} (Green et al., 2010). The aqueous, ethanol and chloroform extracts of seeds and roots showed activity at 1 μg mL^{-1}

against *Bacillus pumulis* (van Vuuren, 2008). These assays were all performed on crude extracts. However, it is known the *Terminalia* species are rich in tannins (Fyhrquist et al., 2014; Manosroi et al., 2013) and that tannins can give false-positive results in enzyme assays.

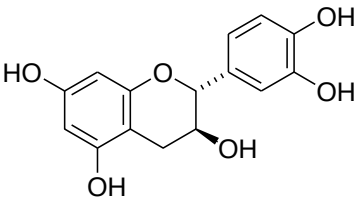
4.1.5 Previous phytochemical investigations

Various phytochemical studies have been conducted on *T. sericea* and several bioactive compounds (mainly triterpenoids and flavonoids) were obtained from different parts of the plant. Table 4.1 presents a summary of the isolated compound in the plant and its activity.

Table 4.1: Bioactive compounds isolated in *T. sericea* (Figure 4.3).

Plant part	**Extract**	**Activity of extract**	**Compound**
Bark	Acetone	Hypoglycaemic and antioxidant activities	Catechin (**4.10**) (Nkobole, 2009)
Bark	Acetone	Hypoglycaemic and antioxidant activities	Epicatechin (**4.11**) (Nkobole, 2009)
Bark	Acetone	Hypoglycaemic and antioxidant activities	Epigallocatechin (**4.12**) (Nkobole, 2009)
Bark	Acetone	Hypoglycaemic and antioxidant activities	Gallocatechin (**4.13**) (Nkobole, 2009)
Bark	Acetone	Hypoglycaemic activity	Lupeol (**4.14**) (Nkobole, 2009)
Bark	Acetone	Hypoglycaemic activity	Sericoside (**4.15**) (Nkobole, 2009)
Bark	Acetone	Hypoglycaemic activity	β-Sitosterol (**4.16**) (Nkobole, 2009)
Bark	Acetone	Hypoglycaemic activity	β-Sitosterol 3-acetate (**4.17**) (Nkobole, 2009)
Root bark	Ethanol	-	Arjungenin (**4.18**) (Joseph et al., 2007)
Root bark	Ethanol	-	Resveratrol 3-β-

			rutinoside (**4.19**) (Joseph et al., 2007)
Root bark	Ethanol	Responsible for the protective ability of wines against coronary heart disease	Resveratrol glucoside (**4.20**) (Joseph et al., 2007)
Root bark	Ethanol	-	Stilbene glycoside (**4.21**) (Joseph et al., 2007)
Root bark	Ethanol	-	Stigmasterol (**4.22**) (Joseph et al., 2007)
Roots	Ethyl acetate	Antibacterial, antifungal, antimalarial and anti-HIV activities (Eldeen, 2008)	Anolignan A (**4.23**) (Eldeen, 2008)
Roots	Ethyl acetate	Antibacterial and anti-inflammatory activities	Anolignan B (**4.24**) (Eldeen et al., 2006)
Roots	Ethyl acetate	activity against Gram-positive and Gram-negative bacteria	Arjunic acid (**4.25**) (Eldeen, 2008)
Roots	Ethyl acetate	-	Arjunglucoside (**4.26**) (Fyhrquist et al., 2006)
Roots	Ethyl acetate	Activity against Gram-positive and Gram-negative bacteria	Termilignan B (**4.27**) (Eldeen, 2008)
Roots	-	Hypoglycaemic activity (Musabayane et al., 2005)	Oleanolic acid (**4.28**) (Eldeen, 2008)



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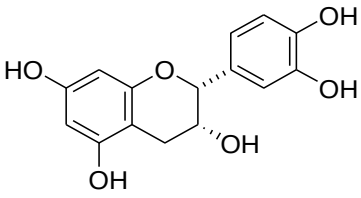

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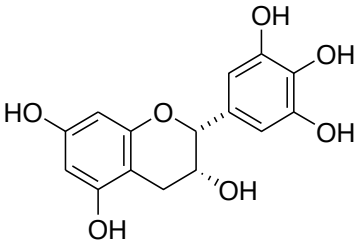

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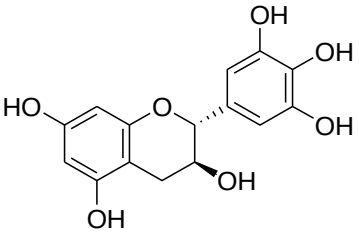
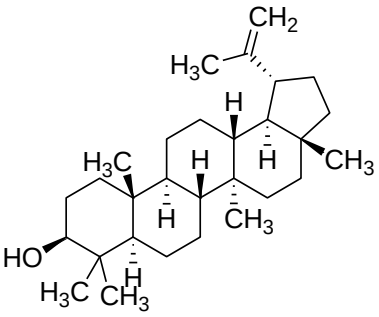
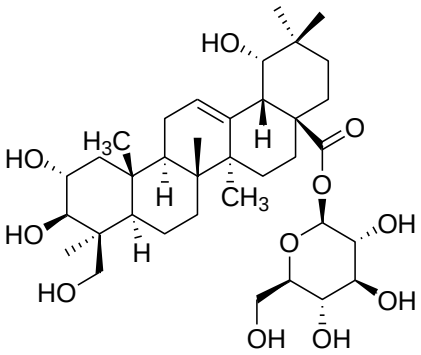


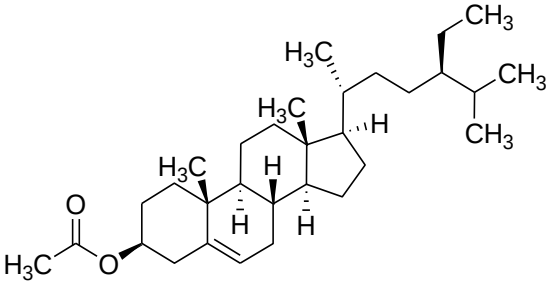
4.13 Gallocatechin



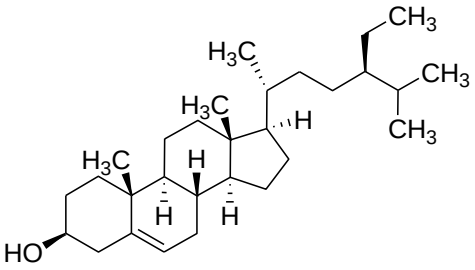
4.14 Lupeol



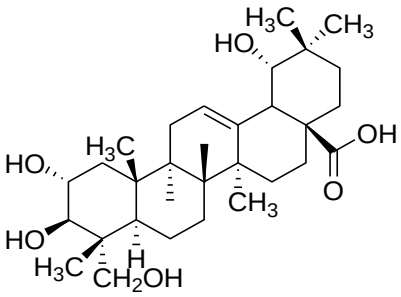
4.15 Sericoside



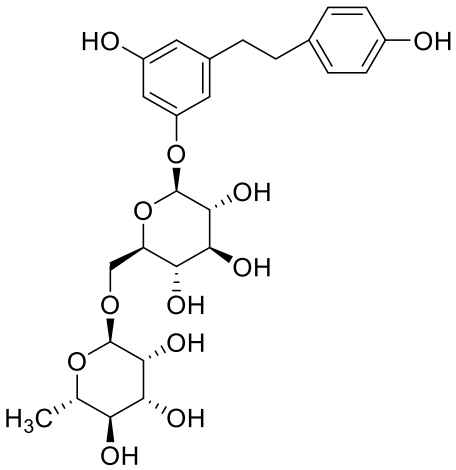
4.16 β-Sitosterol



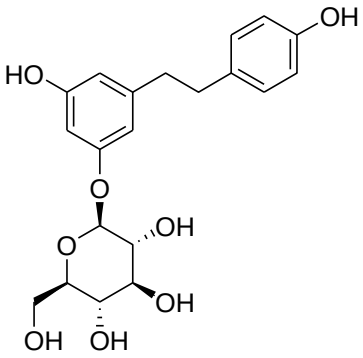
4.17 β-Sitosterol 3-acetate



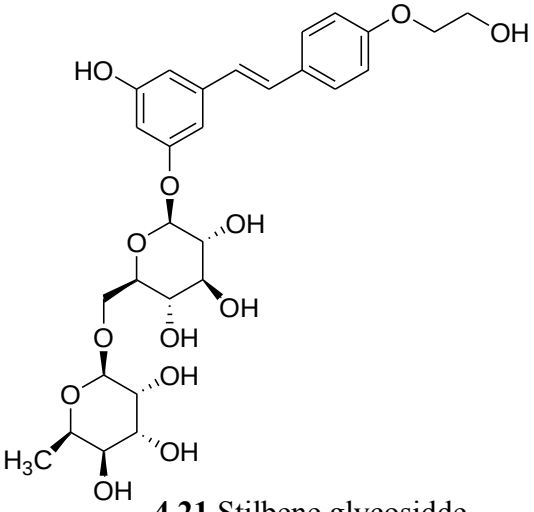
4.18 Arjungenin



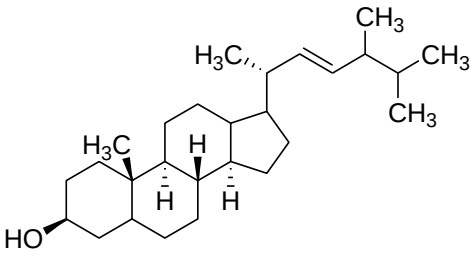
4.19 Resveratrol 3-rutinoside



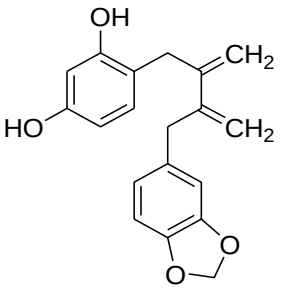
4.20 Resveratrol glucoside



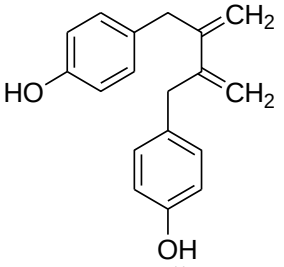
4.21 Stilbene glycosidde



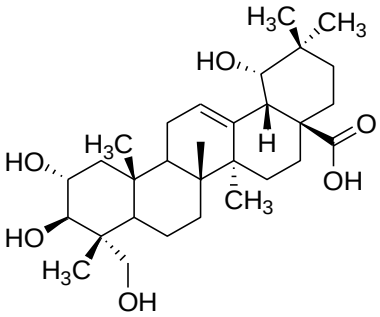
4.22 Stigmasterol



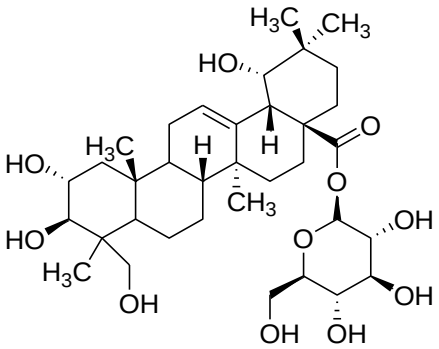
4.23 Anolignan A



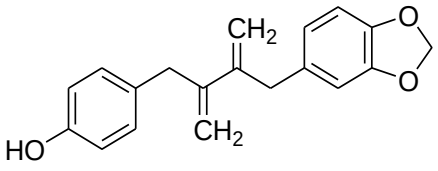
4.24 Anolignan B



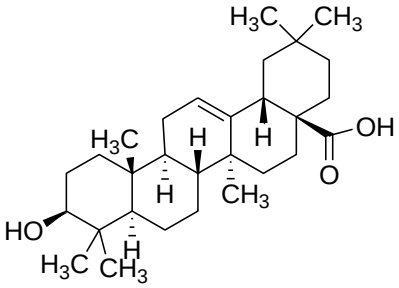
4.25 Arjunic acid



4.26 Arjunglucoside



4.27 Termilignan B



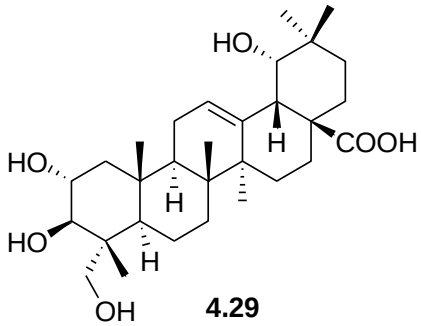
4.28 Oleanolic acid

Figure 4.3: Compounds isolated from *T. sericea*.

4.2 RESULTS AND DISCUSSION

4.2.1 Isolation of sericic acid (4.29)

The MeOH extract of *T. sericea* roots was assayed for antidiabetic activity (see Section 4.2.2). In the assay, antidiabetic activity was observed. A phytochemical analysis resulted in the isolation of one compound (**4.29**). The small amount of extract available did not allow us to isolate and identify more compounds.



4.29

The ^{1}H NMR (Plate 5a) and ^{13}C NMR (Plate 5b) spectra of **4.29** were consistent with an olean-12-ene type pentacyclic triterpene. In the ^{13}C NMR spectrum of **4.29** 29 resonances corresponding to six CH_3 carbons, nine CH_2, seven CH and seven quaternary carbons were observed. The spectrum also showed two olefinic C-atoms at δ_C 124.5 and δ_C 145.0. In the ^{13}C NMR spectrum, four oxygen-containing carbon atoms, three of which were methine carbons resonating at δ_C 85.9, 82.8 and 70.1 and one which was a methylene carbon resonating at δ_C 66.5. The ^{1}H NMR spectrum of **4.29** showed six three-proton singlets at δ_H 0.73, 0.92, 0.95, 0.98, 1.25, and 1.28, which can be assigned to six tertiary methyl groups, and one olefinic proton resonance at δ_H 5.49 (s, H-12). ^{1}H, ^{13}C correlations observed in the HMBC spectrum indicated that the A-ring is oxygenated at C-2 and C-3 as well as on one of the methyl groups on C-4. In Figure 4.4 some of the ^{1}H,^{13}C correlations observed in the HMBC spectrum are shown.

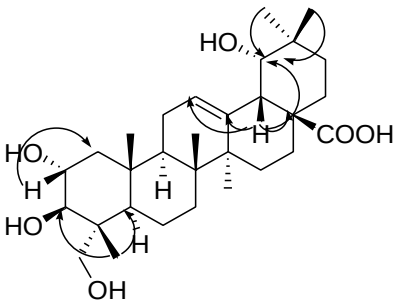


Figure 4.4: Selected HMBC correlations of **4.29**.

Based on the NMR data, the structure of **4.29** was assigned as sericic acid, a triterpenoid isolated from several *Terminalia* species. The NMR assignments correspond to those reported by Ponou et al. (Ponou et al., 2011). In the ^{13}C NMR spectrum we did not observe a resonance for the carboxylic acid carbon, most likely because of a long relaxation time for this carbon. Sericic acid was first reported from *T. sericea* together with its glucosyl ester **4.15** (Bombarde.E et al., 1974). Is known that **4.29** occurs in different species of *Terminalia* and was also isolated in members of other families (Hess and Monache, 1999).

In the next section the effect of the crude MeOH extract of *T. sericea* roots on the glucose utilization of two cell line are described. Unfortunately we did not have enough material of **4.29** to repeat the experiment with the pure compound.

4.2.2 Antidiabetic activity and cytotoxicity of the methanol extract of *T. sericea*

4.2.2.1 Cytotoxicity screening

To evaluate the antidiabetic activity of the MeOH extract (roots) of *T. sericea*, the same experimental procedures as reported for *H. colchicifolia* were used. The first assay determined the viability of liver and muscle cell lines in the presence of the crude extract of *T. sericea*.

The viability of a liver cell line (Chang) was determined by treating the cells with the extract at 12.5, 25 and 50 μg mL^{-1} and the viability was determined at 12, 24 and 48 h (Figure 4.5). A significant reduction in viability was observed at all three concentrations after 24 and 48 h. These results show that the *T. sericea* extract is toxic to this cell line.

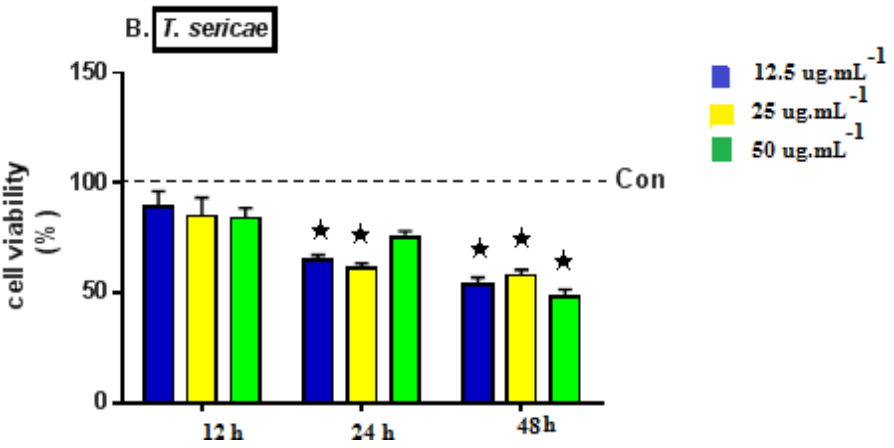


Figure 4.5: Cell viability of liver cell line (Chang) treated with *T. sericea* extract at concentration of 12.5, 25 and 50 µg mL^{-1} after 12, 24 and 48 h. * = $p < 0.05$ in comparison to control.

The experiment was repeated with a muscle cell line (C2C12) and the results are shown in Figure 4.6.

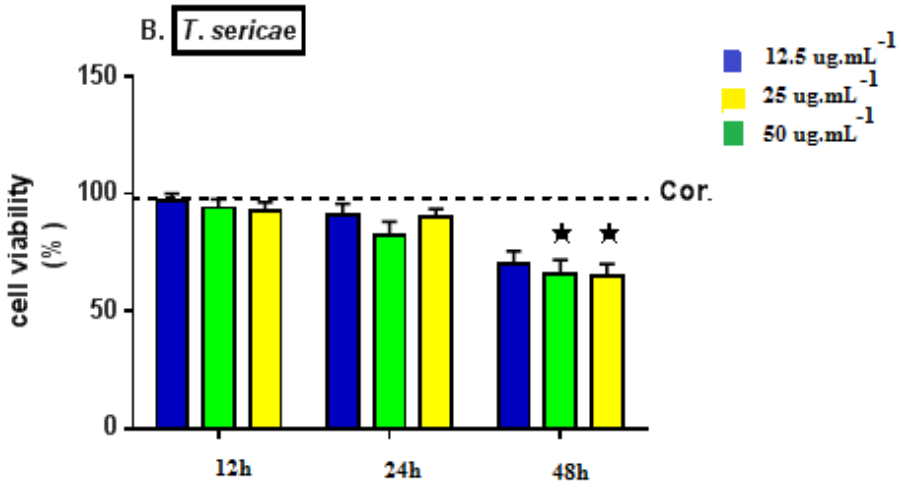


Figure 4.6: Cell viability of muscle cell line (C2C12) treated with *T. sericea* extract at concentrations of 12.5, 25 and 50 µg mL^{-1} after 12, 24 and 48 h. * = $p < 0.05$ in comparison to control.

It is clear that the viability of the muscle cells (C2C12) is affected to a lesser degree by the extract of *T. sericea* than the liver cells, but some cytotoxicity is still observed.

4.2.2.2 Glucose utilization when the cells are treated with the methanol extract of *T. sericea*

The muscle cell line (C2C12) was treated with different concentrations of the extract. The results (Figure 4.7) showed that treatment with the extract resulted in significant reduction in the level of glucose in the medium.

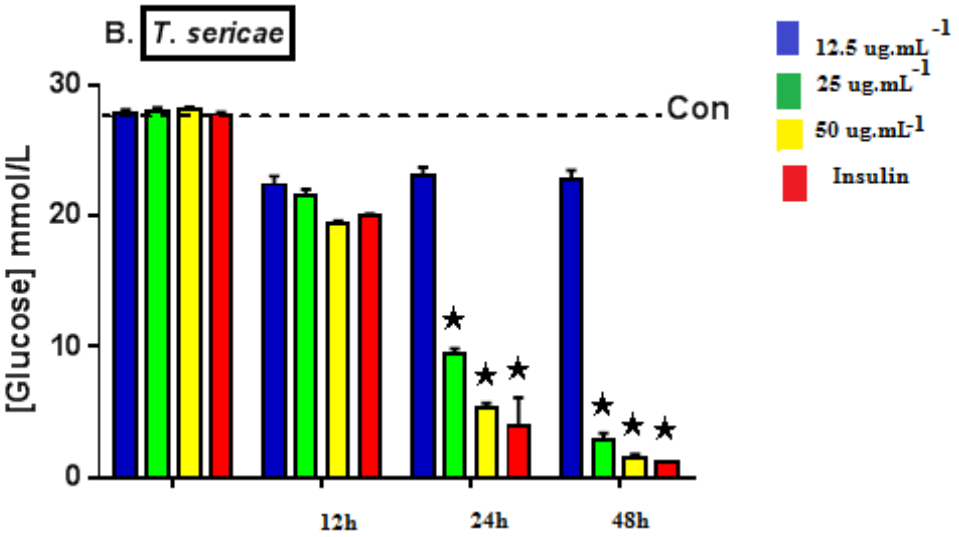


Figure 4.7: Effect of *T. sericea* concentration (12.5, 25 and 50 µg mL^{-1}) on glucose utilization in muscle (C2C12) cell line after 12, 24 and 48 h. * = $p < 0.05$ in comparison to control.

The results of utilization of glucose in muscle cells (C2C12) when treated with concentrations of 12.5, 25 and 50 µg mL^{-1} of extract show that for the muscle cells, a significant reduction in the level of glucose in concentrations of 25-50 µg mL^{-1} was observed, while for a concentration 12.5 µg mL^{-1} the glucose level remains constant after 24 h. These results indicated that the extract increases the glucose utilization of the C2C12 muscle cell line and that the effect was dose dependent.

The experiment was repeated with a liver cell line (Chang) and the results are shown in Figure 4.8.

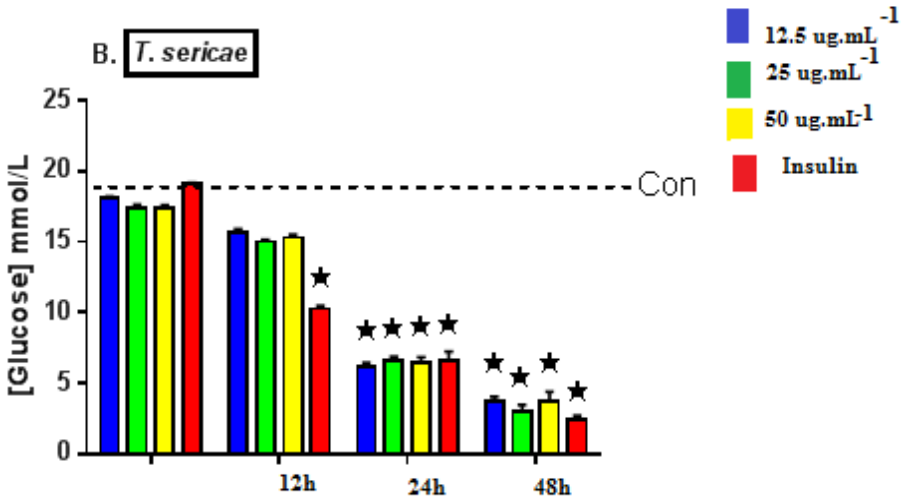


Figure 4.8: Effect of the *T. sericea* extract (12.5, 25 and 50 µgmL^{-1}) on glucose utilization in liver cell line (Chang) after 12, 24 and 48 h. * = $p < 0.05$ in comparison to control.

For liver cells (Chang) the results of the glucose utilization when the cells are treated with the MeOH extract of *T. sericea* (roots) show that regardless of the concentration, there is a significant reduction of the level of glucose after 24 h. This is an indication that the extract caused a significant increase in the glucose utilization in both the C2C12 muscle cells and Chang liver cells which indicated that it has antidiabetic activity.

4.3 CONCLUSION

In the current phytochemical study, one known compound (**4.29**) was isolated from the methanol extract of *T. sericea* roots.

The MeOH extract of *T. sericea* roots exhibited *in vitro* hypoglycaemic activity which validates the utilization of *T. sericea* roots to treat diabetes in traditional medicine. However, it can be concluded from the analysis of results that the MeOH extract of *T. sericea* roots also show some cytotoxicity toward both cell lines.

This is the first study that reports *in vitro* antidiabetic activity for *T. sericea* roots. Previously Nkobole (2009) reported that the acetone extract *T. sericea* (stem bark) had inhibitory activity against α-amylase enzyme (90%) and α-glycosidase (97%) (Nkobole, 2009).

Future studies are needed in order to determine the mechanism by which the root extract of *T. sericea* has hypoglycaemic activity and to test the activity of the isolated compounds.

4.4 EXPERIMENTAL

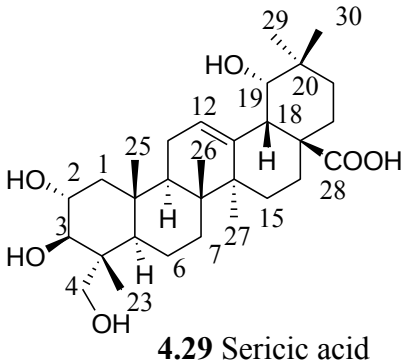
4.4.1 Collection of plant material

T. sericea roots were collected in Maputo National Reserve, Maputo Province, Mozambique. The plant was identified by Antonio Mapanga (botanist in Department of Biological Science at the University Eduardo Mondlane, Mozambique) and a voucher (T.S. 205) was deposited in the Herbarium of the Department of Biological Science at the University Eduardo Mondlane, Mozambique. The plant material was air-dried in the shade at room temperature for 2 weeks. It was then ground with a mechanical mill, affording a mass of 103 g.

The root powder (103 g) was extracted successively with DCM and MeOH, for 24 h each at room temperature. After each extraction, the solvent was filtered using Whatman® No 1 filter paper and concentrated under vacuum using a rotary evaporator. The extracts were then dried under high vacuum. The yields were 2.6 g for the DCM extract and 4.2 g for the MeOH extract.

4.4.2 Extraction and isolation of sericic acid (4.29)

The dry MeOH extract (2.5 g) was diluted with H_2O and the dark solution was extracted with DCM and then with *n*-BuOH. Both extracts were evaporated under vacuum to dryness, to yield 135 mg and 636 mg for the DCM and *n*-BuOH extract, respectively (adapted from Bombarde et al., 1974). The BuOH extract was concentrated, diluted with diethyl ether and a brown solid collected. The BuOH extract was subjected to column chromatography with the mobile phase MeOH: EtOAc (1:19) to yield 2.1 mg of compound **29.4**.



4.29 Sericic acid

4.4.3 Physical properties of sericic acid (4.29)

Sericic acid (**4.29**) was isolated as a white solid substance. ^{1}H NMR (400 MHz, CD_3OD): δ_H 5.49 (1H, s, H-12); 4.03 (1H, d. *J* = 11.2 Hz, H_a-24); 3.80 (1H, ddd, *J* = 11.0, 9.5, 4.4 Hz, H-2); 3.39 (1H, d, *J* = 11.2 Hz, H_b-24); 3.26 (1H, d, *J* = 4.0 Hz, H-19); 3.07 (1H, d, *J* = 4.0 Hz, H-18); 3.06 (1H, d, *J* = 9.5 Hz, H-3); 1.99-1.96 (2H, m, H-11); 1.93 (1H, dd, *J* = 12.0 Hz, 4.5, H_{eq}-1); 1.80-1.74 (1H, m, H_a-7); 1.79-1.74 (1H, m, H_a-21); 1.75-1.67 (1H, m, H-9); 1.65-1.60 (1H, m, H_b-7); 1.64-1.60 (1H, m, H_a-15); 1.64-1.59 (1H, m, H_a-6); 1.50-1.45 (1H, m, H_a-22); 1.46-1.42 (1H, m, H_b-6); 1.34-1.30 (1H, m, H_b-22); 1.28 (3H, s, H-27); 1.25 (3H, s, H-23); 1.02-0.98 (1H, m, H_b-21); 1.00-0.97 (1H, m, H_b-15); 0.98 (3H, s, H-25); 0.99 - 0.96 (1H, m, H-5); 0.95 (3H, s, H-30); 0.92 (3H, s, H-29), 0.90 (1H, dd, *J* = 12.0 and 10.9 Hz, H_{ax}-1); 0.73 (3H, s, H-26). ^{13}C NMR (100 MHz, CD_3OD): δ_C 145.0 (C-13); 124.3 (C-12); 85.9 (C-3); 82.8 (C-19); 70.1 (C-2); 66.5 (C-24); 57.6 (C-5); 49.1 (C-9); 48.1 (C-1); 47.1 (C-17); 44.9 (C-18); 44.3 (C-4); 43.0 (C-14); 41.2 (C-8); 39.1 (C-10); 35.9 (C-20); 34.4 (C-22); 33.9 (C-7); 29.9 (C-15); 29.6 (C-21); 28.9 (C-29); 28.5 (C-16); 25.2 (C-30) and (C-11); 25.0 (C-27); 24.1 (C-23); 20.0 (C-6); 17.9 (C-26); 17.4 (C-25).

CHAPTER 5: GENERAL CONCLUSIONS

In most plants, the active compounds are secondary metabolites. These compounds are responsible for protecting plants against microbial infections or infestations by pests (Doughari and Rao, 2012; Sarker et al., 2006). Natural products isolated from plants and microorganisms have played a vital role as a source of lead compounds for the pharmaceutical industry.

In this project, the phytochemical and antidiabetic activity of two medicinal plants from South Africa namely *Hypoxis colchicifolia* Bak (Hypoxidaceae) and *Terminalia sericea* Burch. (Combretaceae) were studied.

H. colchicifolia (*H. latifolia*) is after *H. hemerocallidea* (African Potato) the second most important *Hypoxis* medicinal species with commercial value in South Africa. From the methanol extract of the corms four phenolic derivatives, hypoxoside, a mixture of dehydroxyhypoxoside A and B and bis-dehydroxyhypoxoside were isolated as well as an environmental impurity, bisphenol A diglycidyl ether. This is the first report on the isolation of dehydroxyhypoxoside B. The results showed that the MeOH extract of *H. colchicifolia* decreased the level of glucose and it has low toxicity *in vitro*.

T. sericea is an important plant in traditional medicine and is in the top 50 most important African medicinal plants. From the methanol extract of the roots one known compound, sericic acid, was isolated. *T. sericea* exhibited *in vitro* hypoglycaemic activity, but also showed some cytotoxicity.

These results validate the use of *H. colchicifolia* and *T. sericea* to treat diabetes in traditional medicine. Future studies are needed to determine the mechanism by which both plants present the hypoglycaemic activity. There are many plants used for the treatment of diabetes, but it is particularly important to pay attention to the toxicity of some species.

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APPENDIX: NMR, IR, UV AND HR-MS SPECTRA OF THE ISOLATED COMPOUNDS

Plate 1a: ^{1}H NMR spectrum of hypoxoside (**3.1**) in CD_3OD.

Plate 1b: ^{13}C NMR spectrum of hypoxoside (**3.1**) in CD_3OD.

Plate 1c: COSY NMR spectrum of hypoxoside (**3.1**) in CD_3OD.

Plate 1d: HSQC NMR spectrum of hypoxoside (**3.1**) in CD_3OD.

Plate 1e: HMBC NMR spectrum of hypoxoside (**3.1**) in CD3OD.

Plate 1f: IR spectrum of hypoxoside (**3.1**).

Plate 1g: UV spectrum of hypoxoside (**3.1**) in MeOH.

Plate 1h: High-resolution mass spectrum of hypoxoside (**3.1**).

Plate 2a: ^{1}H NMR spectrum of dehydroxyhypoxoside A (**3.14**) and B (**3.15**) in CD_3OD.

Plate 2b: ^{13}C NMR spectrum of dehydroxyhypoxoside A (**3.14**) and B (**3.15**) in CD_3OD.

Plate 2c: COSY NMR spectrum of dehydroxyhypoxoside A (**3.14**) and B (**3.15**) in CD_3OD.

Plate 2d: HSQC NMR spectrum of dehydroxyhypoxoside A (**3.14**) and B (**3.15**) in CD_3OD.

Plate 2e: HMBC NMR spectrum of dehydroxyhypoxoside A (**3.14**) and B (**3.15**) in CD_3OD.

Plate 1f: DEPT NMR spectrum of hypoxoside (3.1) in CD_3OD

Plate 2g: IR spectrum of dehydroxyhypoxoside A (**3.14**) and B (**3.15**).

Plate 2h: UV spectrum of dehydroxyhypoxoside A (**3.14**) and B (**3.15**) in MeOH.

Plate 2i: High-resolution mass spectrum of dehydroxyhypoxoside A (**3.14**) and B (**3.15**).

Plate 3a: ^{1}H NMR spectrum of bis-dehydroxyhypoxoside (**3.16**) in CD_3OD.

Plate 3b: ^{13}C NMR spectrum of bis-dehydroxyhypoxoside (**3.16**) in CD_3OD.

Plate 3c: COSY NMR spectrum of bis-dehydroxyhypoxoside (**3.16**) in CD_3OD.

Plate 3d: HSQC NMR spectrum of bis-dehydroxyhypoxoside (**3.16**) in CD_3OD.

Plate 3e: HMBC NMR spectrum of bis-dehydroxyhypoxoside (**3.16**) in CD_3OD.

Plate 2i: DEPT NMR spectrum of dehydroxyhypoxoside A (3.14) and B (3.15) in CD_3OD

Plate 3g: IR spectrum of bis-dehydroxyhypoxoside (**3.16**).

Plate 3h: UV spectrum of bis-dehydroxyhypoxoside (**3.16**) in MeOH.

Plate 3i: High-resolution mass spectrum of bis-dehydroxyhypoxoside (**3.16**).

Plate 4a: ^{1}H NMR spectrum of bisphenol A diglycidyl ether (**3.17**) in $CDCl_3$.

Plate 4b: ^{13}C NMR spectrum of bisphenol A diglycidyl ether (**3.17**) in $CDCl_3$.

Plate 4c: COSY NMR spectrum of bisphenol A diglycidyl ether (**3.17**) in $CDCl_3$.

Plate 4d: HSQC NMR spectrum of bisphenol A diglycidyl ether (**3.17**) in $CDCl_3$.

Plate 4e: HMBC NMR spectrum of bisphenol A diglycidyl ether (**3.17**) in $CDCl_3$.

Plate 4f: High-resolution mass spectrum bisphenol A diglycidyl (**3.17**).

Plate 5a: ^{1}H NMR spectrum of sericic acid (**4.29**) in CD_3OD.

Plate 5b: ^{13}C NMR spectrum of sericic acid (**4.29**) in CD_3OD.

Plate 5c: HSQC NMR spectrum of sericic acid (**4.29**) in CD_3OD.

Plate 5d: HMBC NMR spectrum of sericic acid (**4.29**) in CD_3OD.

Plate 6a: ^1H NMR spectrum of the fraction one (F1) in CD_3OD

Publications and conferences presentations resulting from this thesis.

Plate 1a: ^{1}H NMR spectrum of hypoxoside (3.1) in CD_3OD.

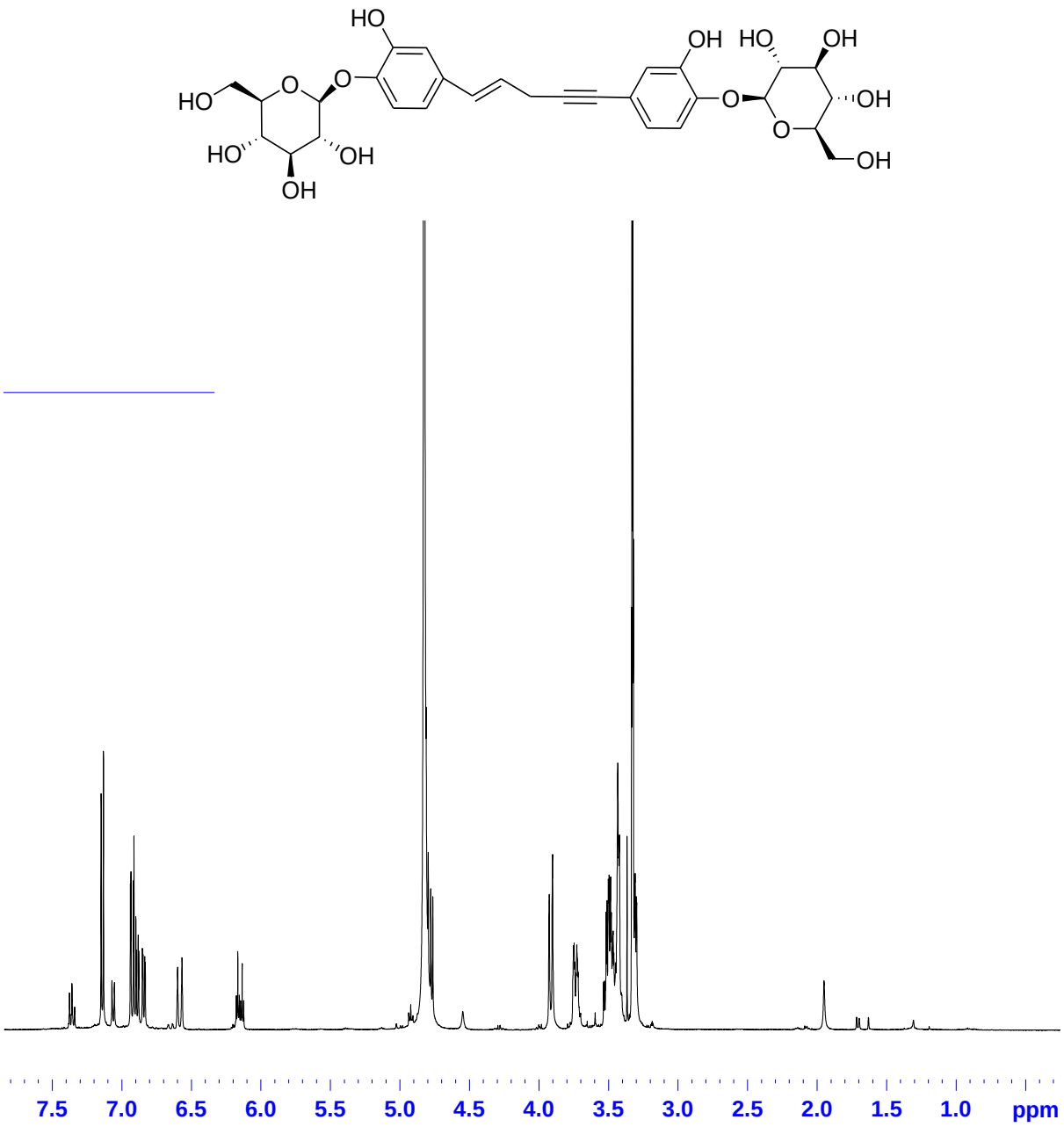


Plate 1b: ^{13}C NMR spectrum of Hypoxoside (3.1) in CD_3OD.

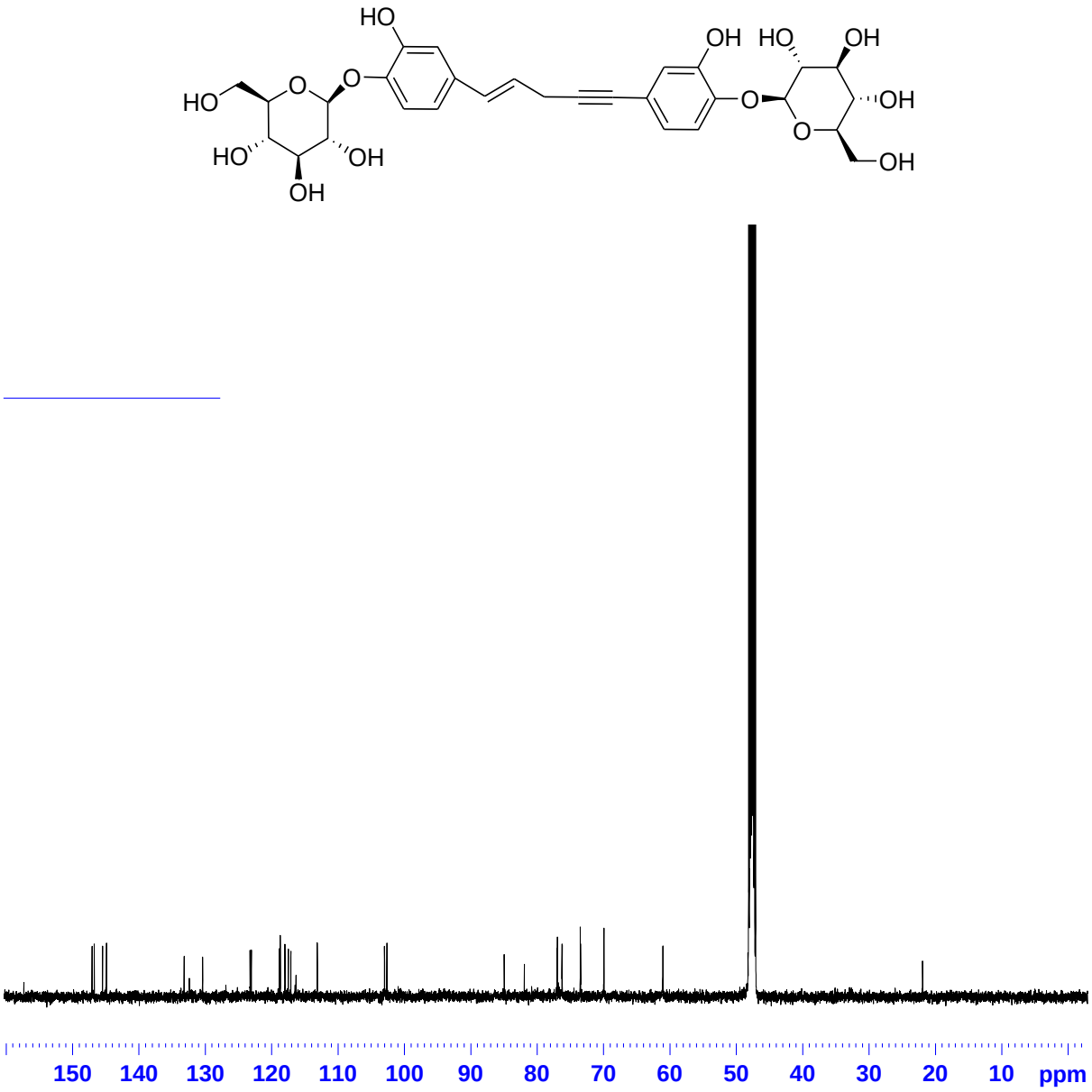


Plate 1c: COSY NMR spectrum of hypoxoside (3.1) in CD_3OD.

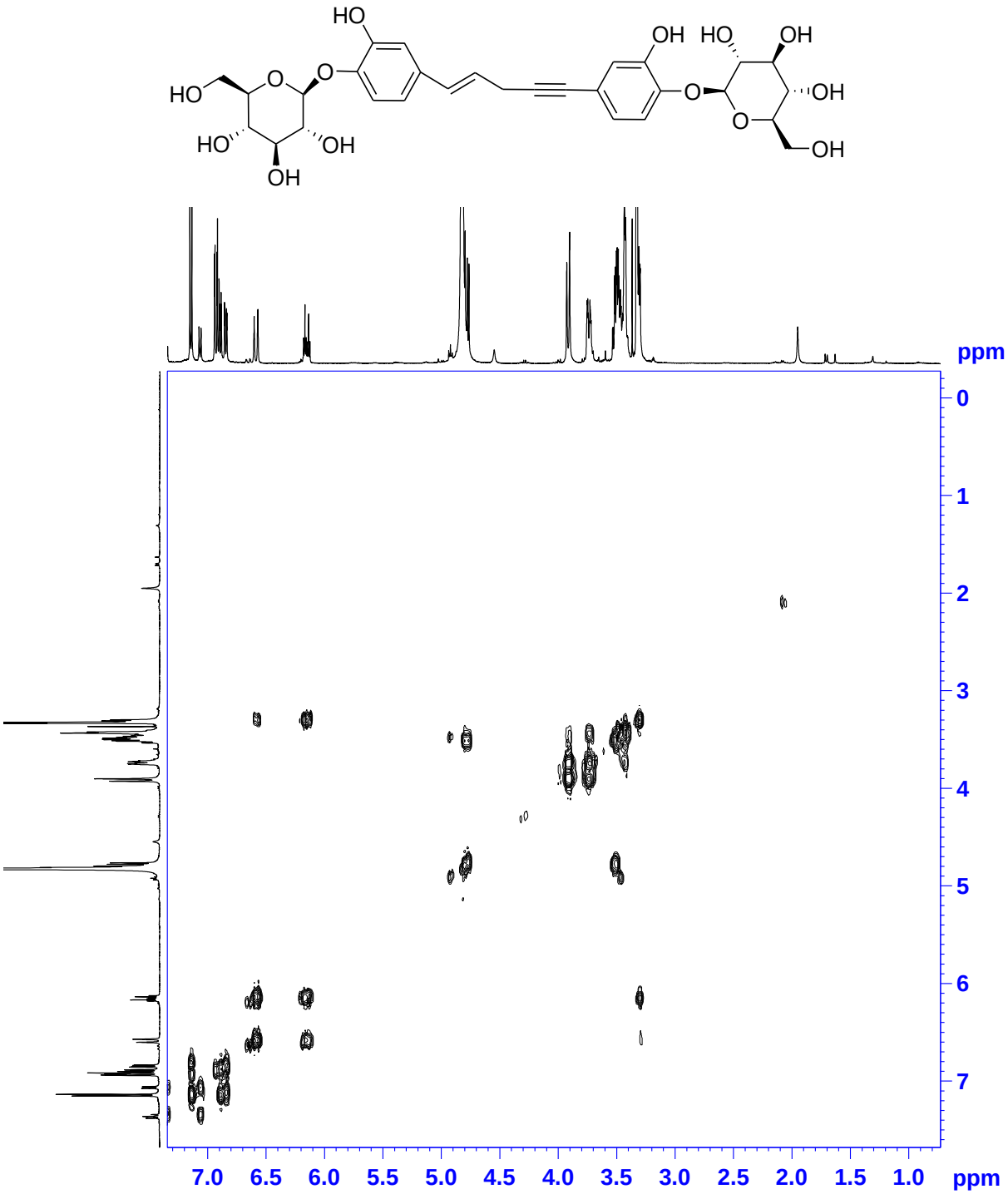


Plate 1d: HSQC NMR spectrum of hypoxoside (3.1) in CD_3OD.

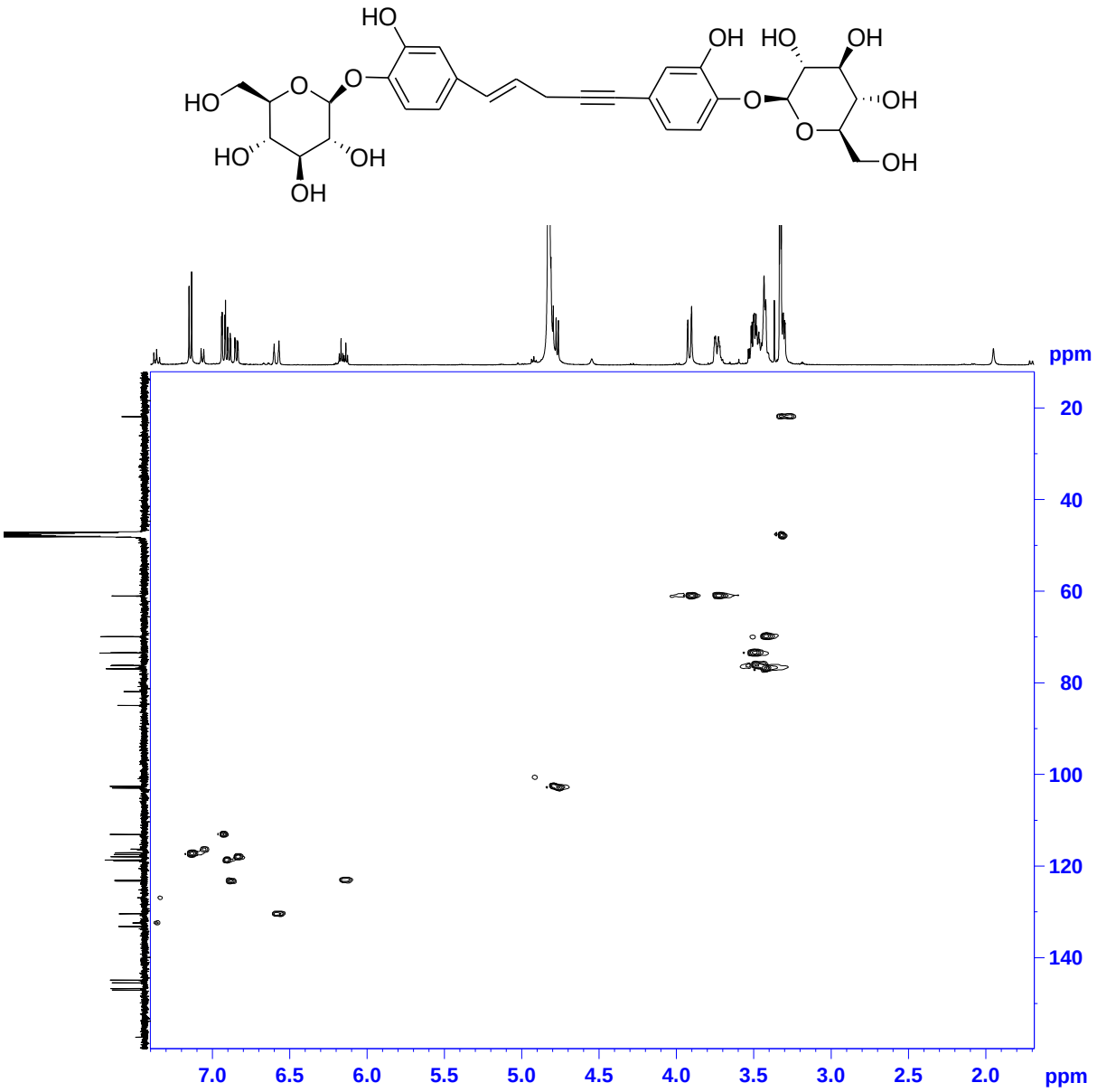


Plate 1e: HMBC NMR spectrum of hypoxoside (3.1) in CD3OD.

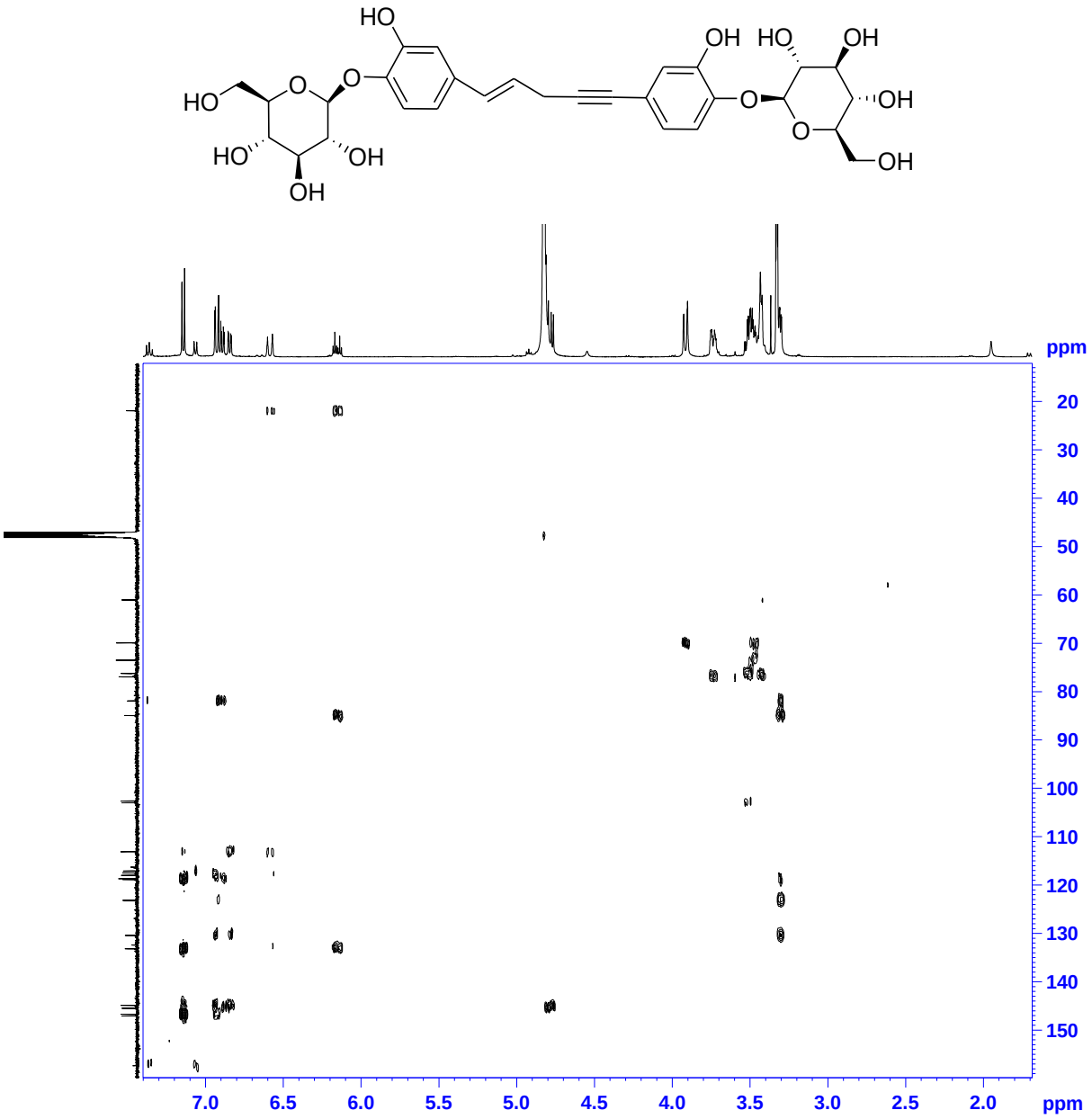


Plate 1f: DEPT NMR spectrum of hypoxoside (3.1) in CD_3OD

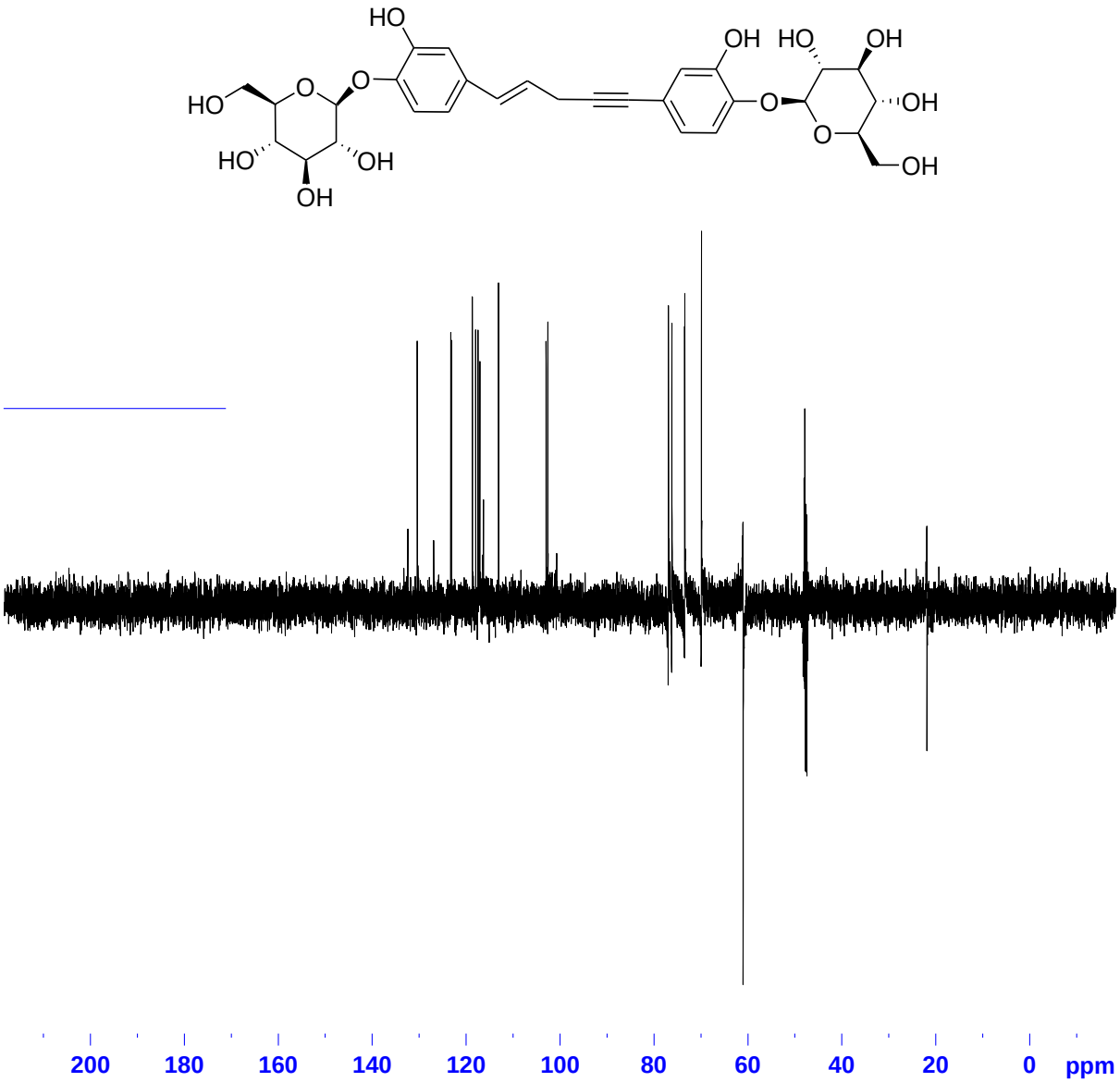
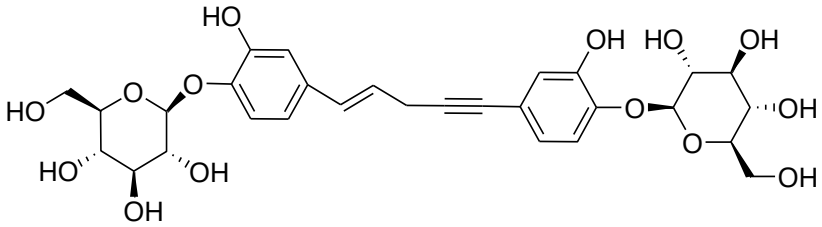


Plate 1g: IR spectrum of hypoxoside (3.1).



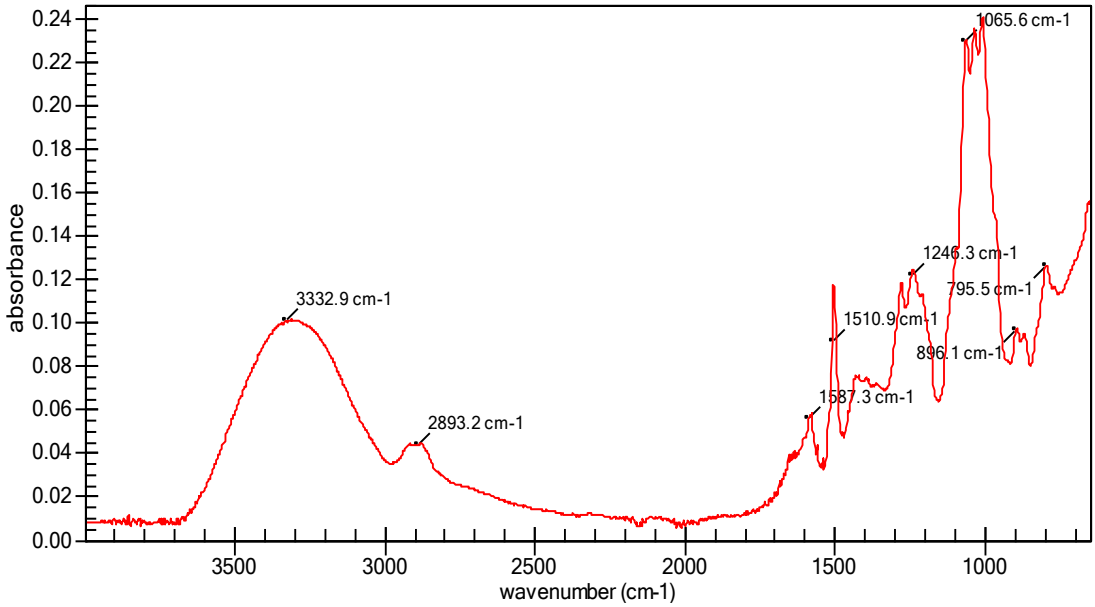
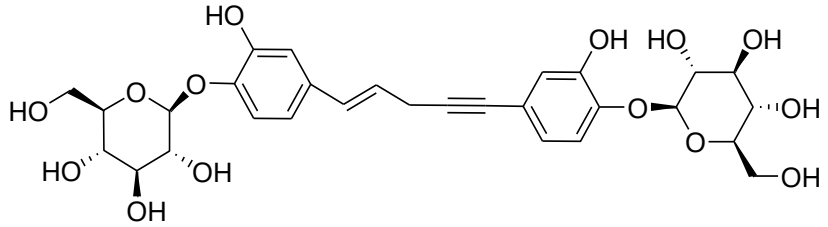


Plate 1h: UV spectrum of hypoxoside (3.1) in MeOH.



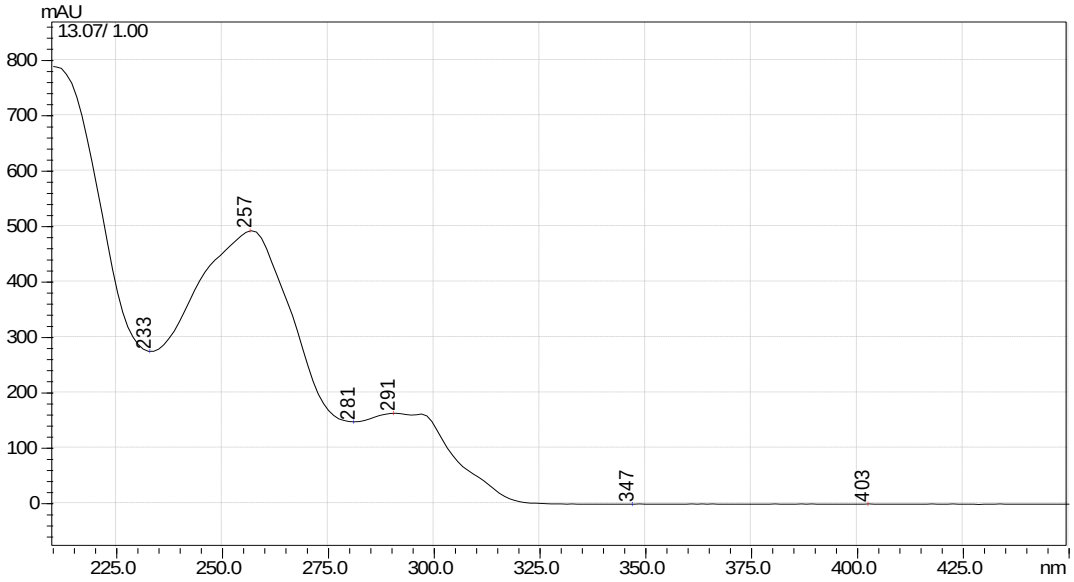
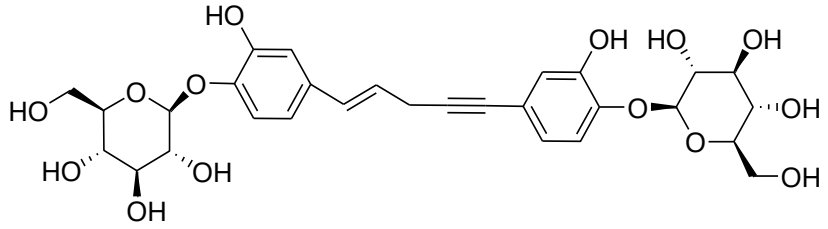
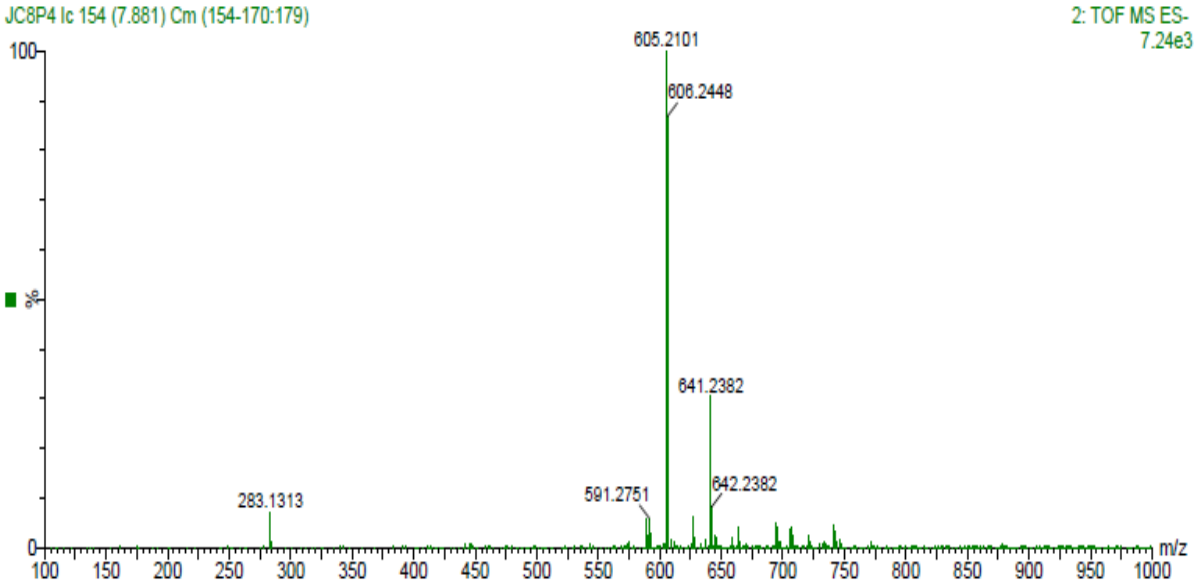
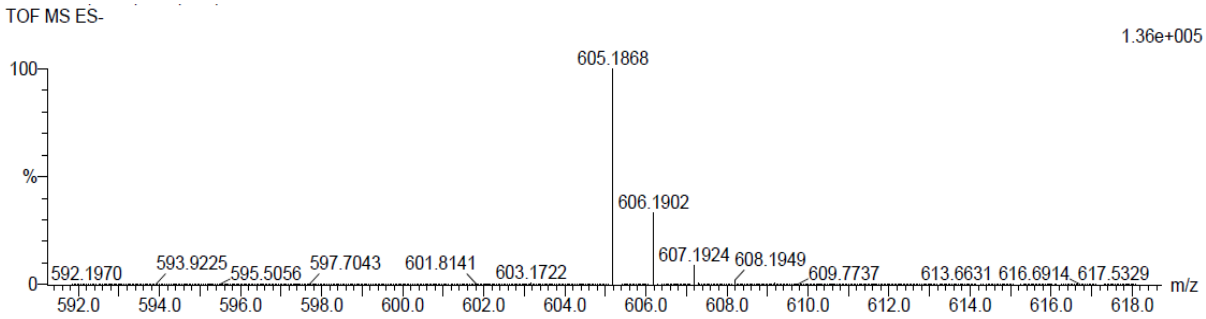


Plate 1i: High-resolution mass spectrum (ESI⁻) of hypoxoside (3.1).







Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
605.1868	605.1870	-0.2	-0.3	13.5	496.9	0.0	C29 H33 O14

Plate 2a: ^{1}H NMR spectrum of dehydroxyhypoxoside A (3.14) and B (3.15) in CD_3OD.

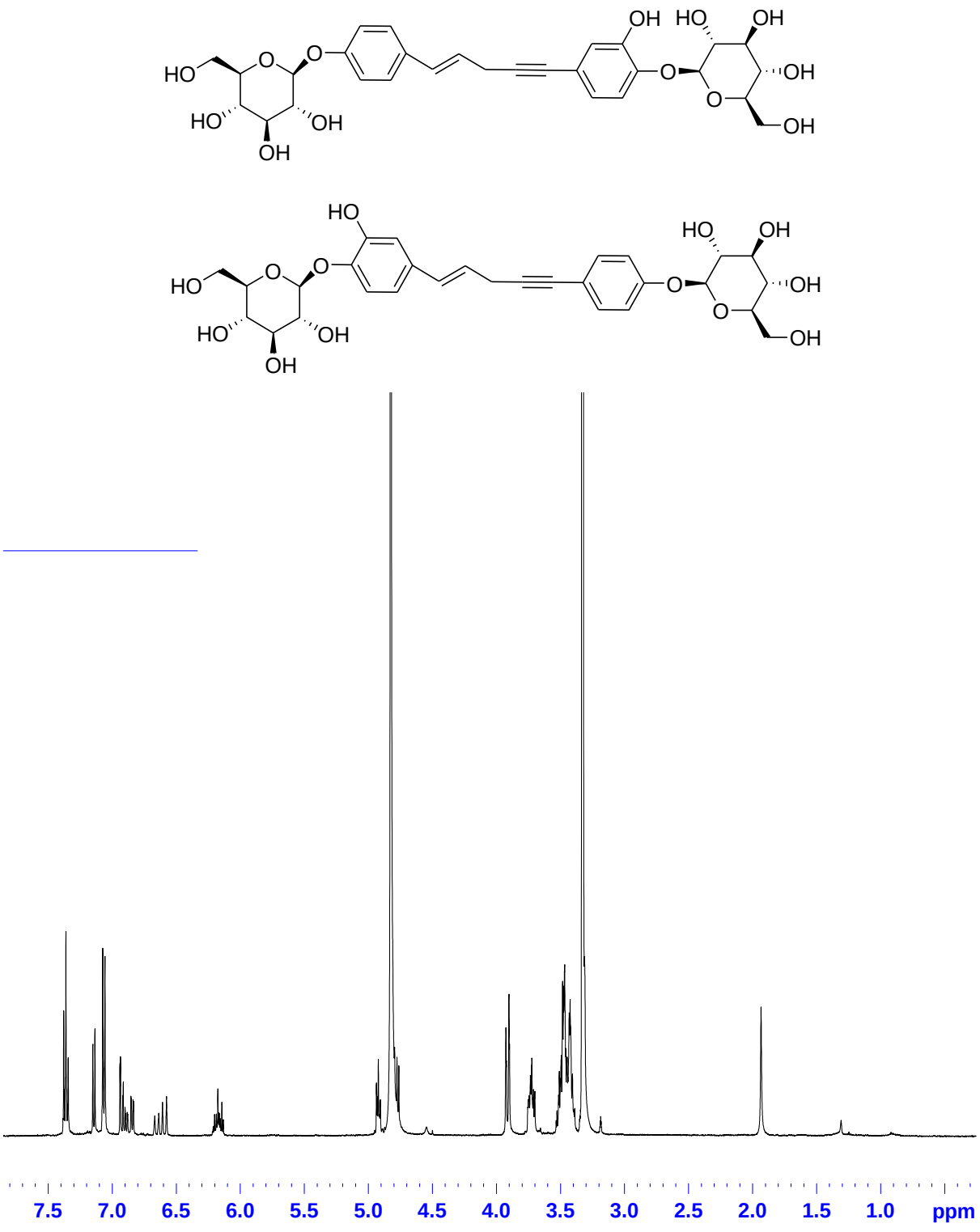


Plate 2b: ^{13}C NMR spectrum of dehydroxyhypoxoside A (3.14) and B (3.15) in CD_3OD.

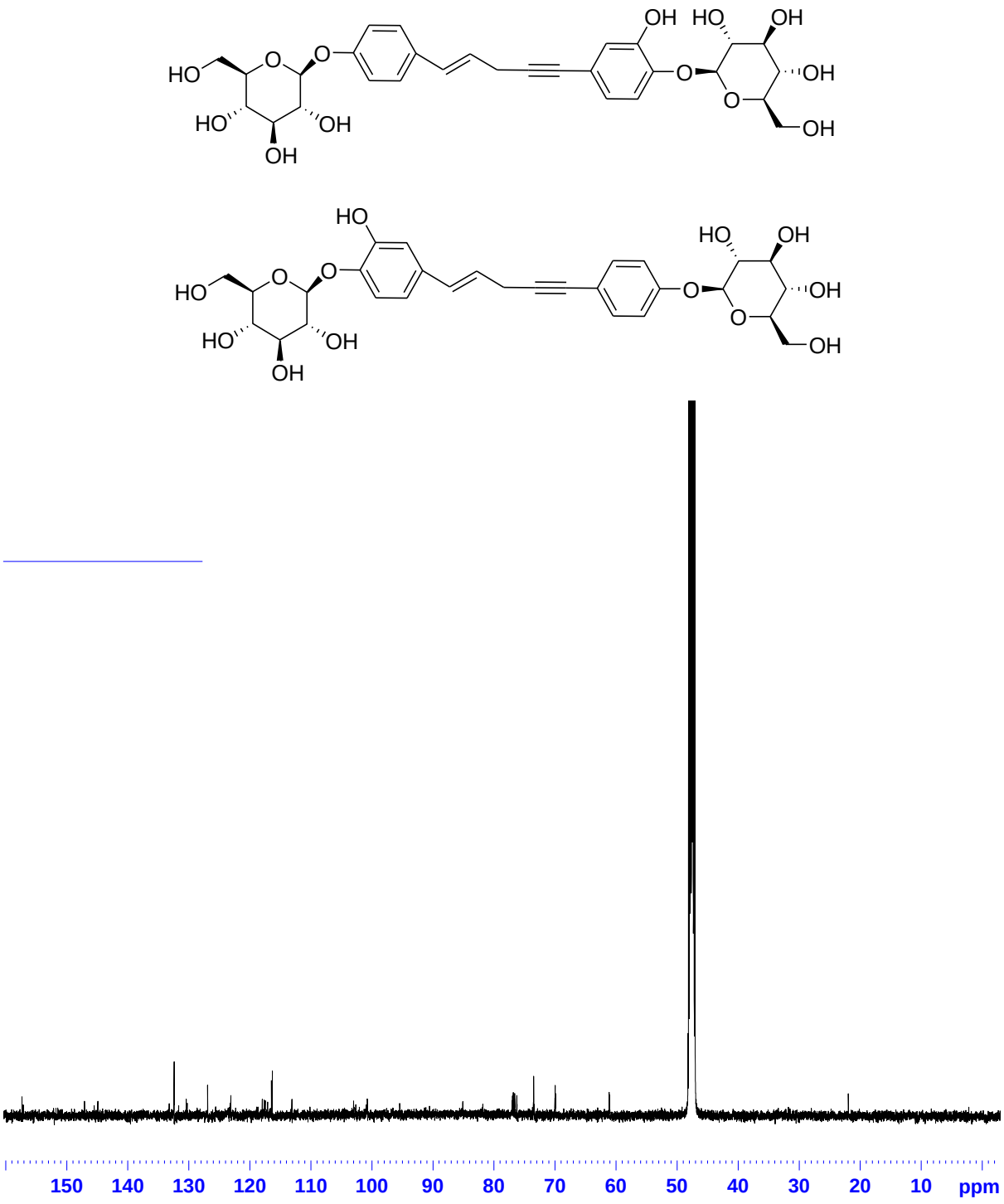


Plate 2c: COSY NMR spectrum of dehydroxyhypoxoside A (3.14) and B (3.15) in CD_3OD.

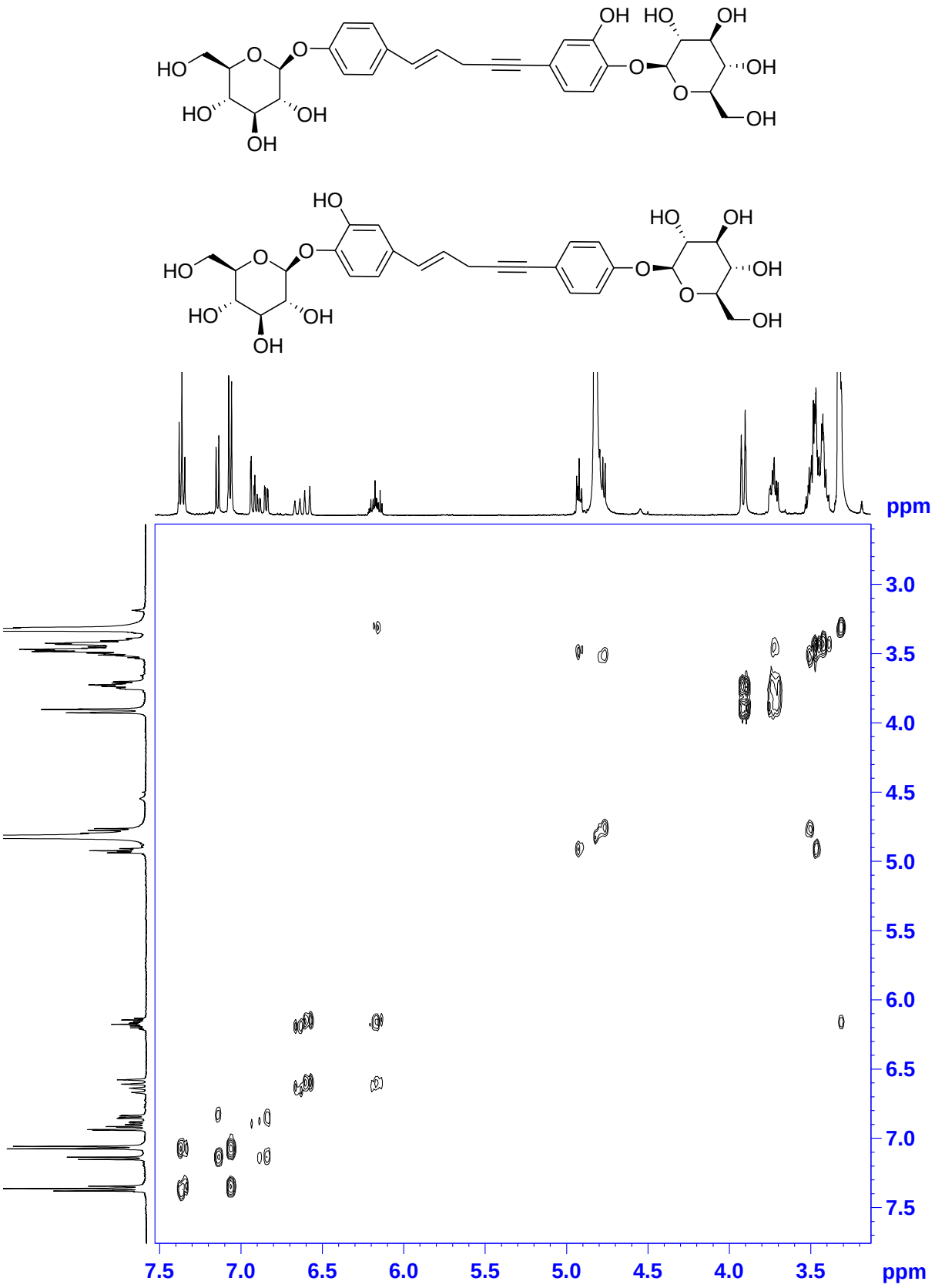


Plate 2d: HSQC NMR spectrum of dehydroxyhypoxoside A (3.14) and B (3.15) in CD_3OD.

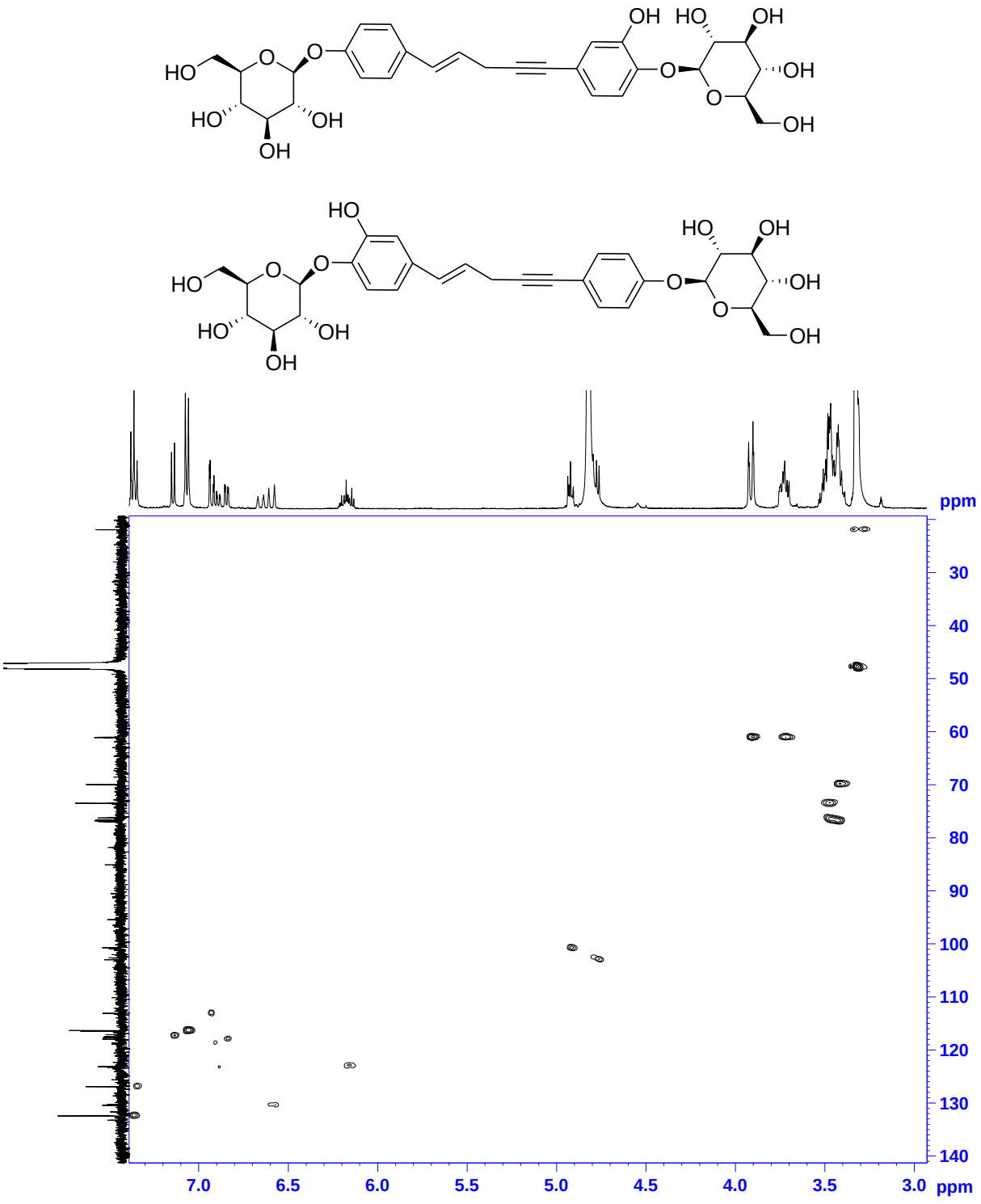


Plate 2e: HMBC NMR spectrum of dehydroxyhypoxoside A (3.14) and B (3.15) in CD_3OD.

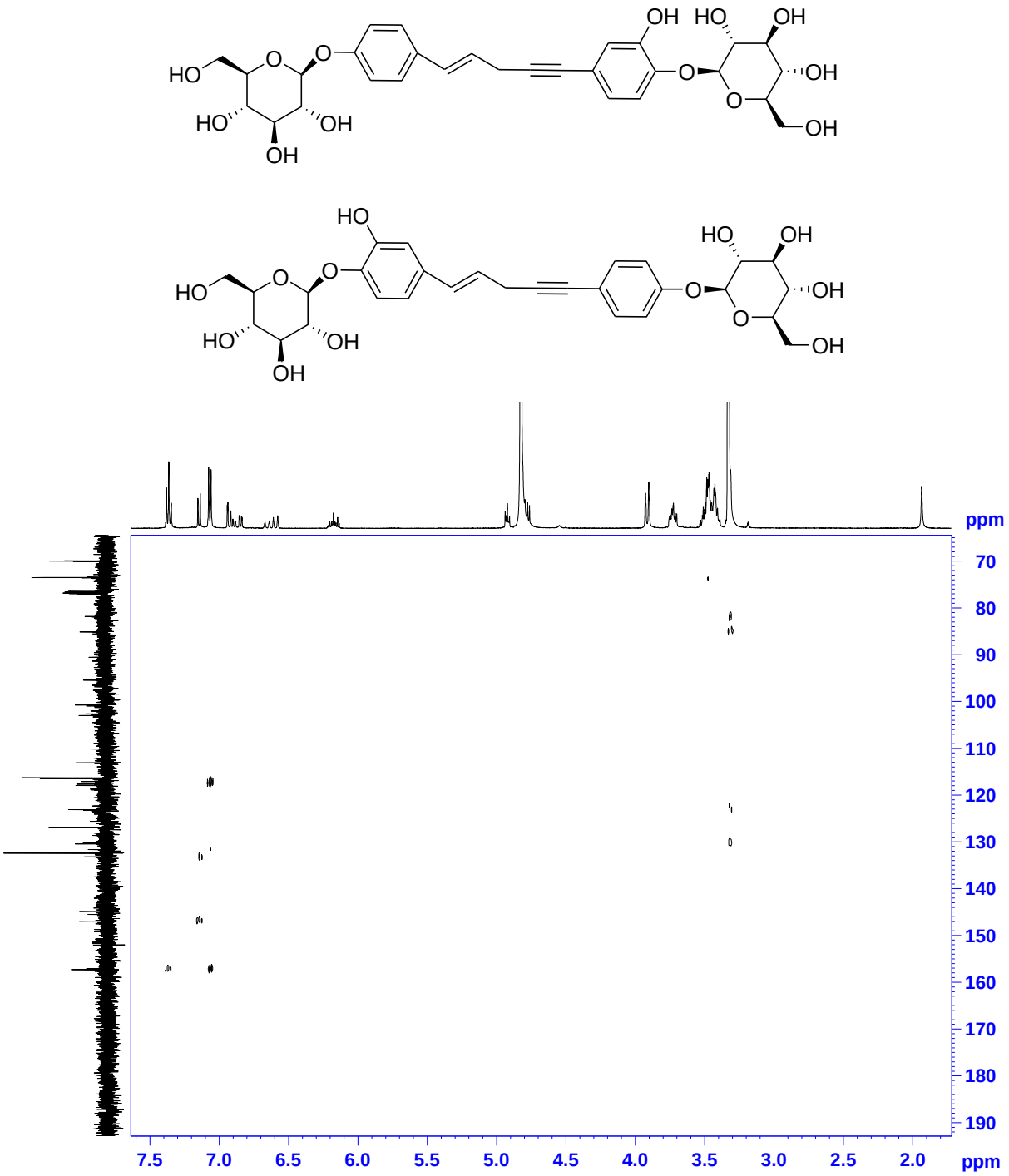


Plate 2i: DEPT NMR spectrum of dehydroxyhypoxoside A (3.14) and B (3.15) in CD_3OD

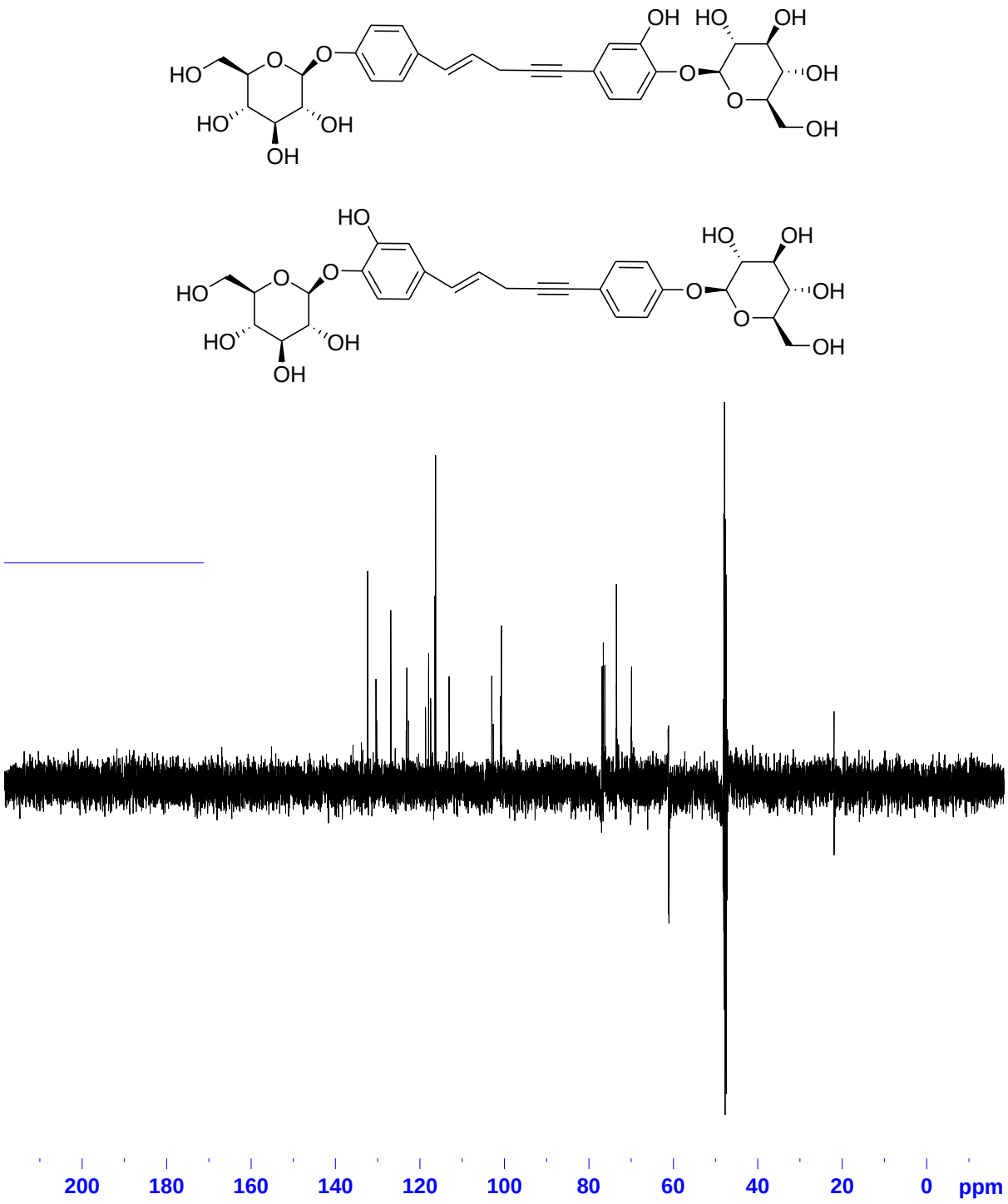
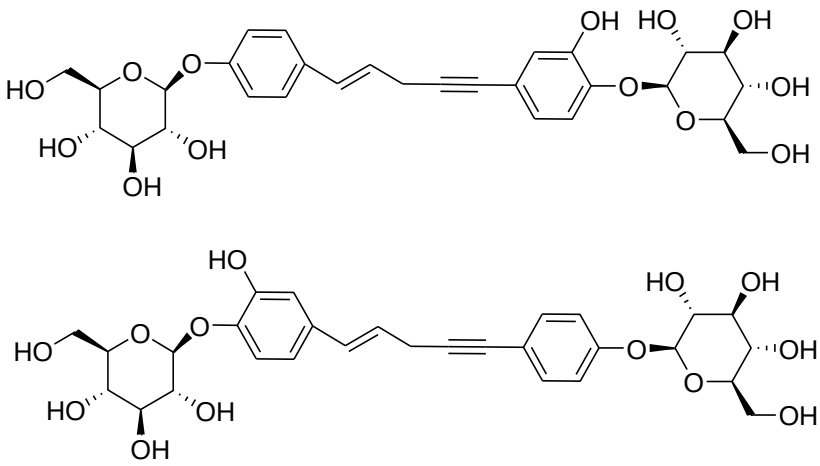


Plate 2g: IR spectrum of dehydroxyhypoxoside A (3.14) and B (3.15).



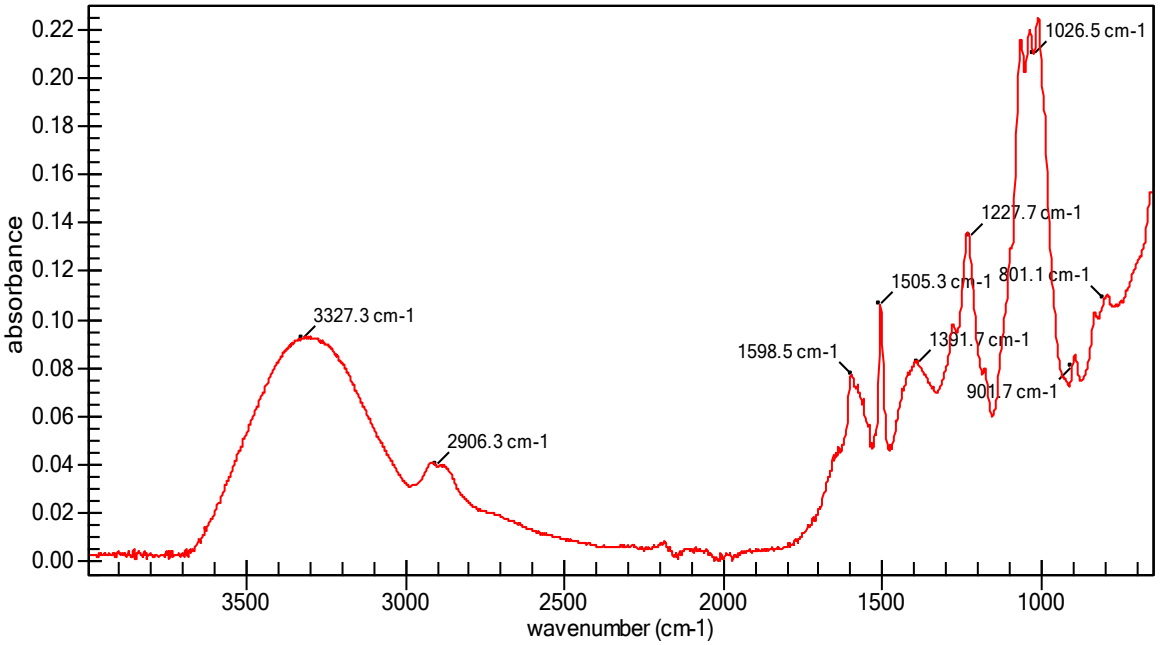
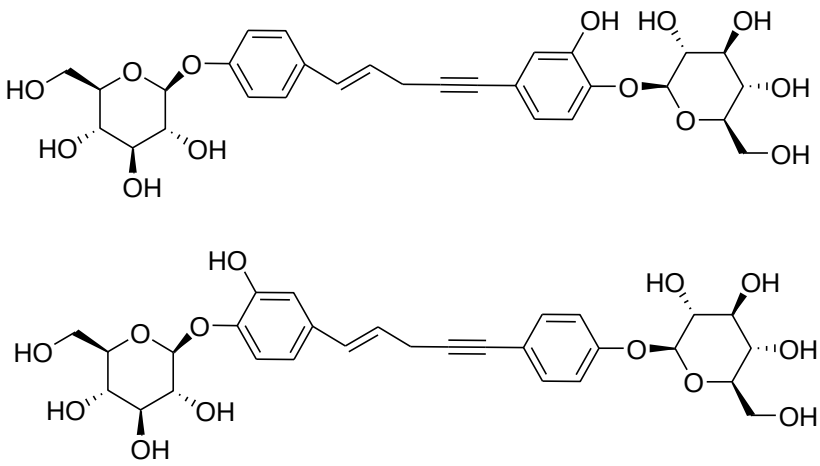


Plate 2h: UV spectrum of dehydroxyhypoxoside A (3.14) and B (3.15) in MeOH.



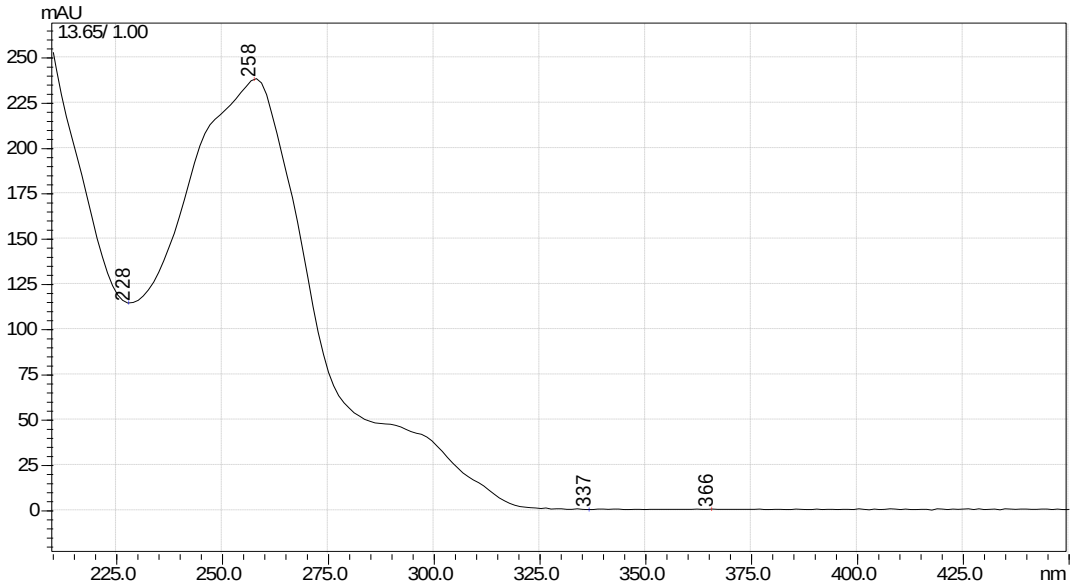
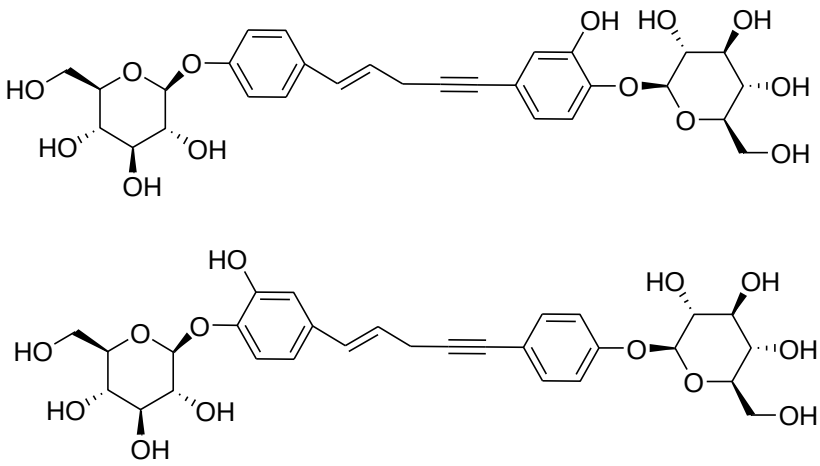
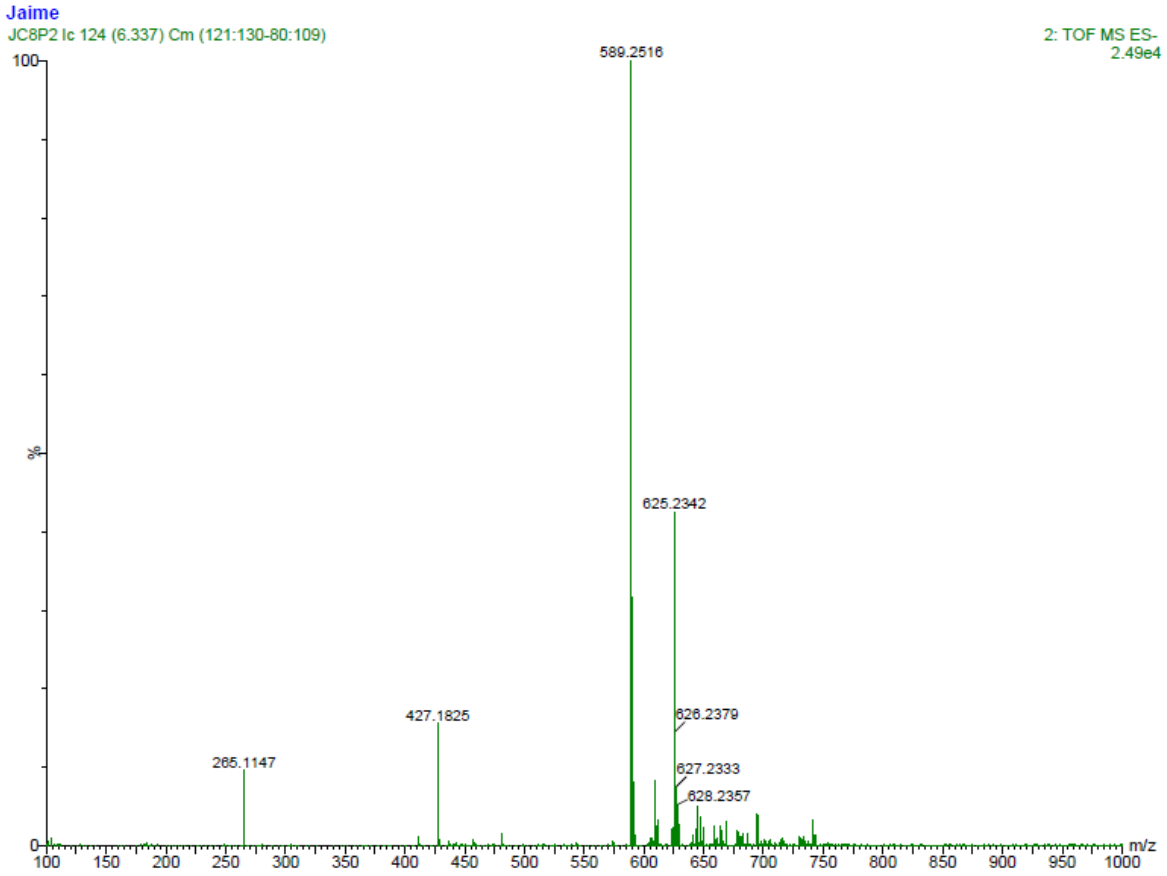
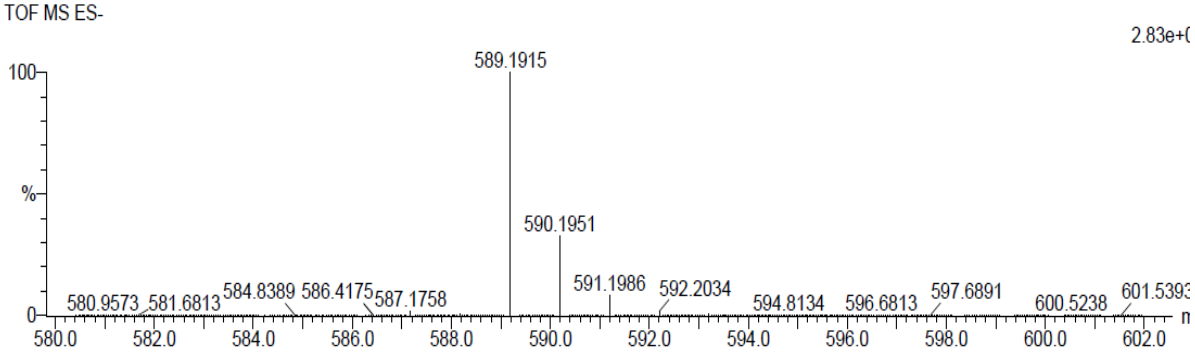


Plate 2i: High-resolution mass spectrum of dehydroxyhypoxoside A (3.14) and B (3.15).





TOF MS ES-



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
589.1915	589.1921	-0.6	-1.0	13.5	520.9	0.0	C29 H33 O13

Plate 3a: ^{1}H NMR spectrum of bis-dehydroxyhypoxoside (3.16) in CD_3OD.

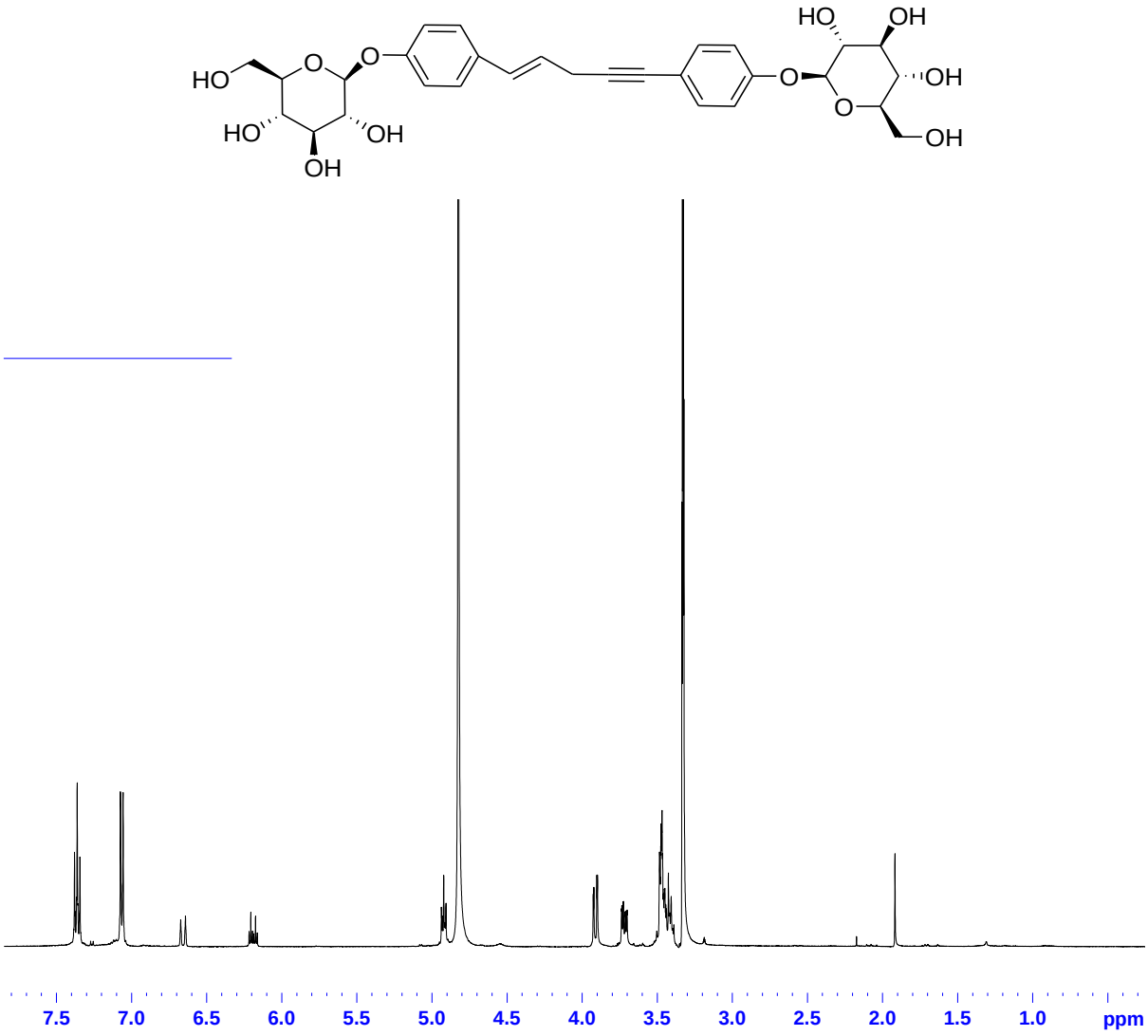


Plate 2b: ^{13}C NMR spectrum of bis-dehydroxyhypoxoside (3.16) in CD_3OD.

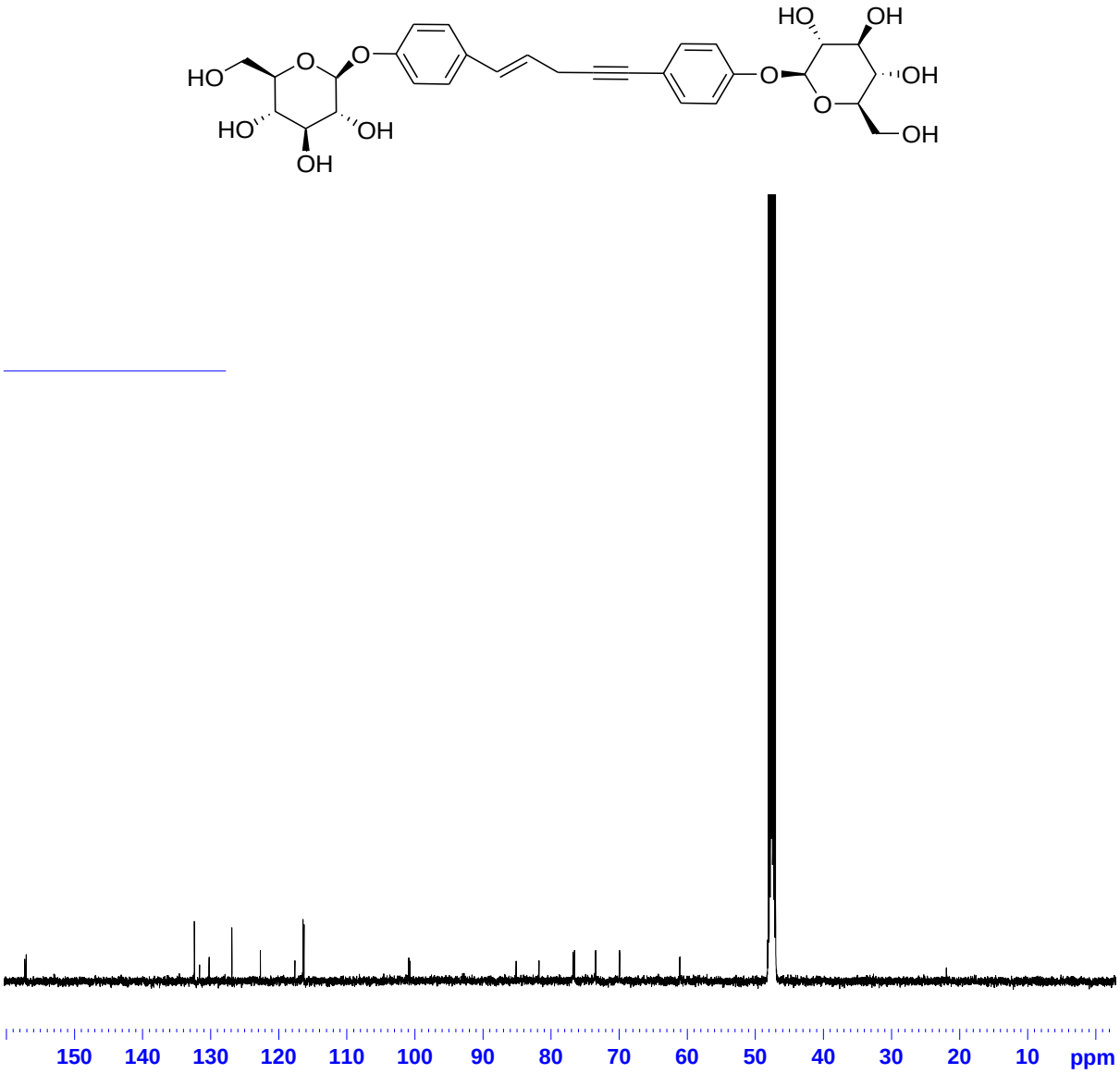


Plate 3c: COSY NMR spectrum of bis-dehydroxyhypoxoside (3.16) in CD_3OD.

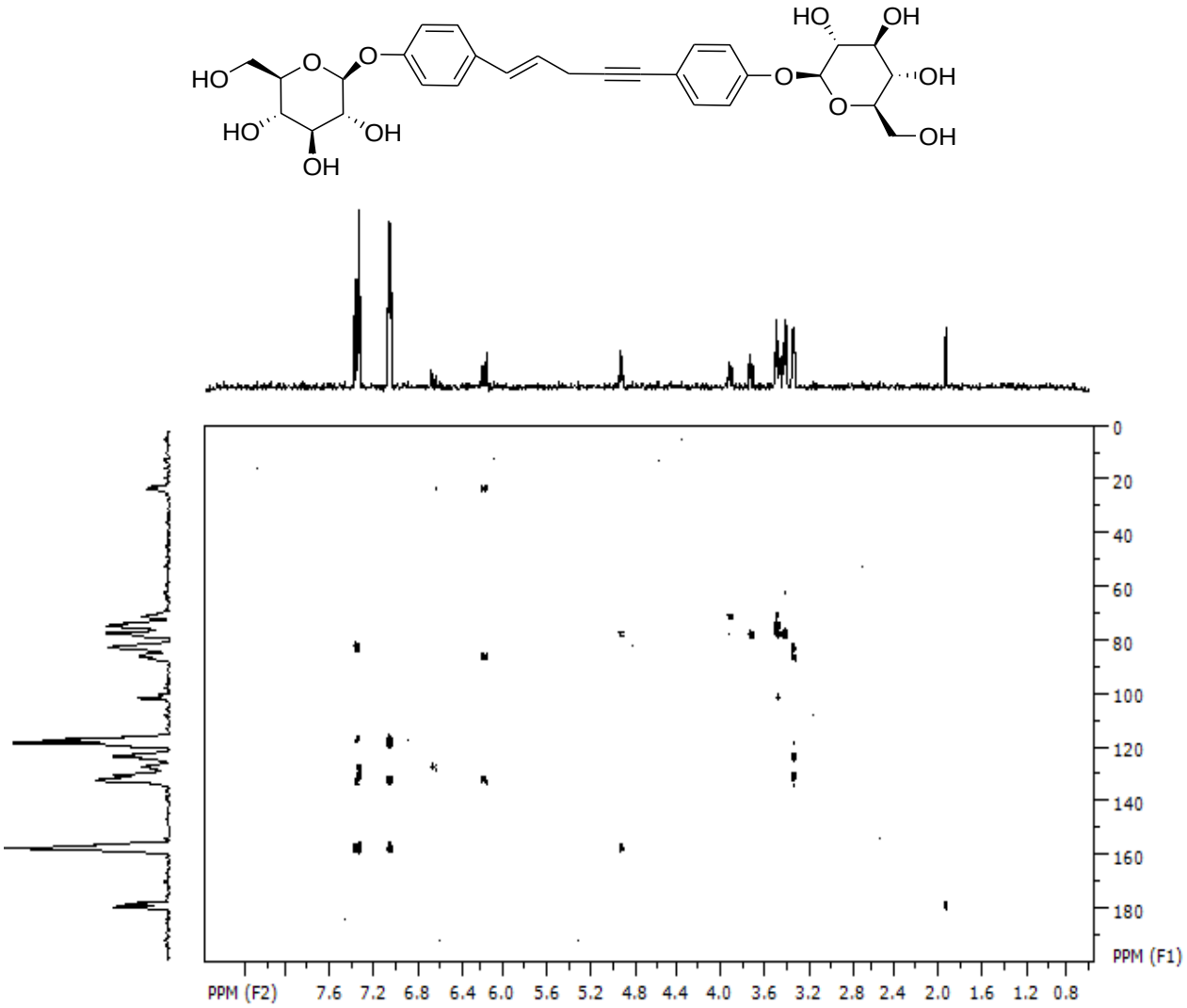


Plate 3d: HSQC NMR spectrum of bis-dehydroxyhypoxoside (3.16) in CD_3OD.

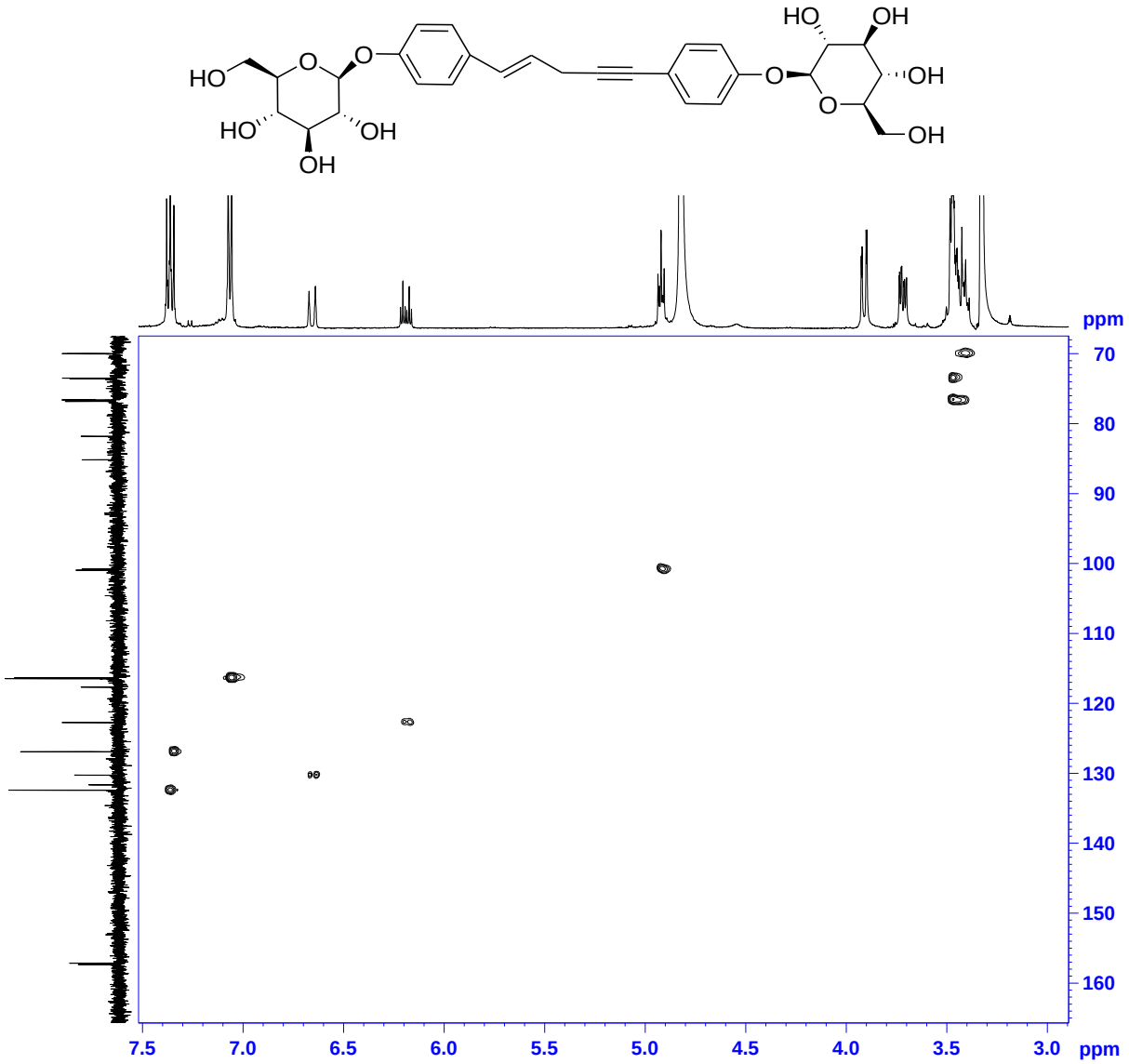


Plate 3e: HMBC NMR spectrum of bis-dehydroxyhypoxoside (3.16) in CD_3OD.

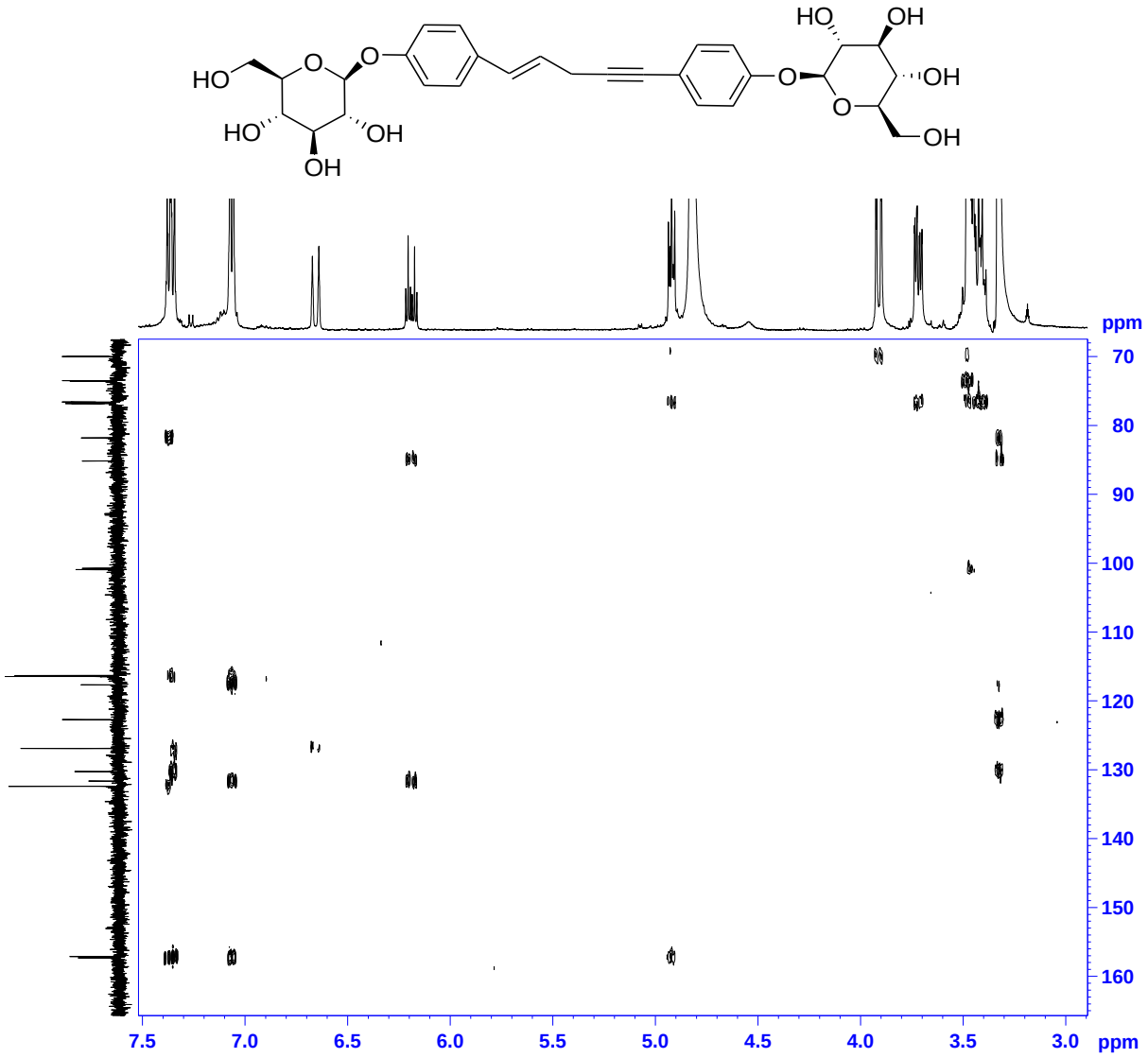
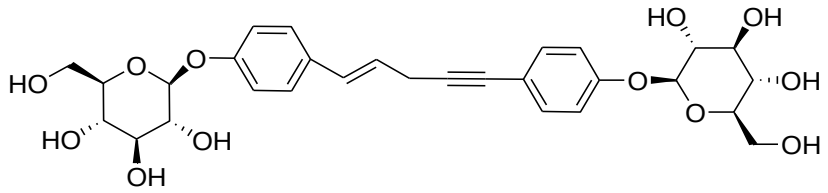


Plate 3f: IR spectrum of bis-dehydroxyhypoxoside (3.16).



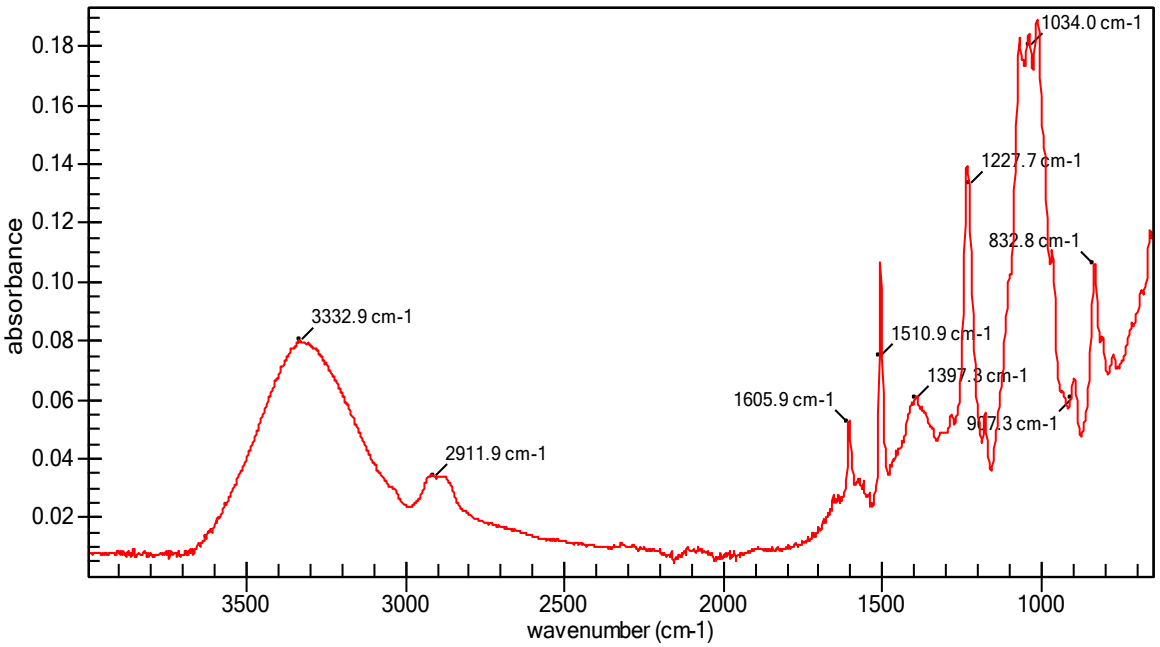
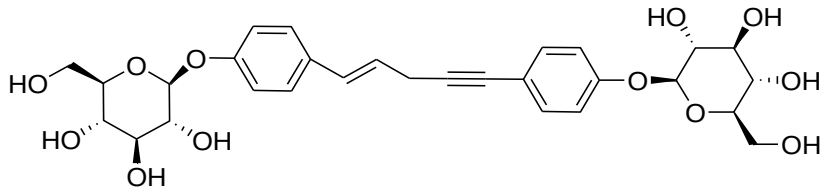


Plate 3g: UV spectrum of bis-dehydroxyhypoxoside (3.16) in MeOH.



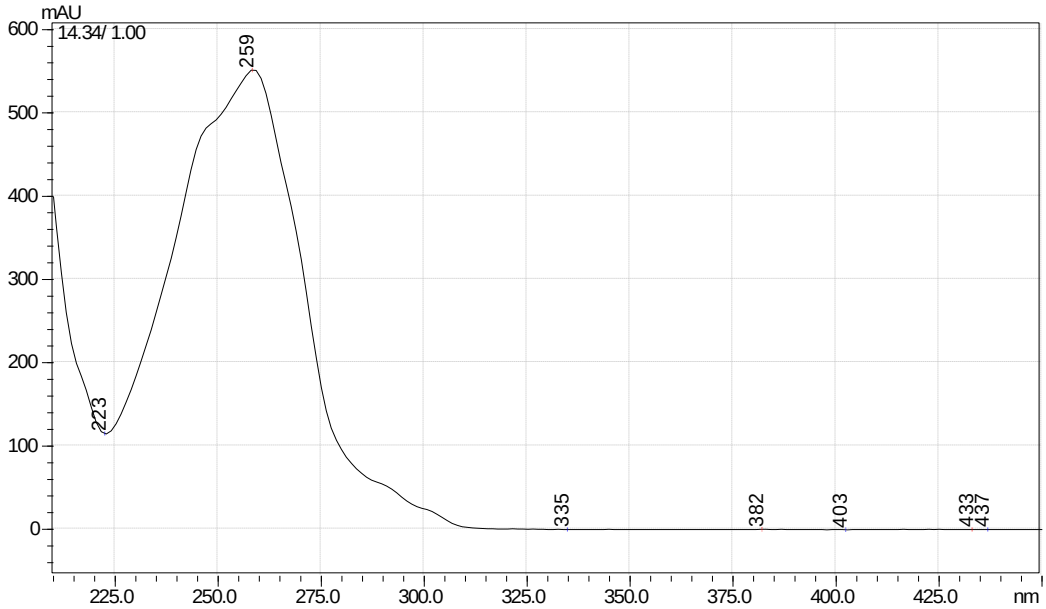
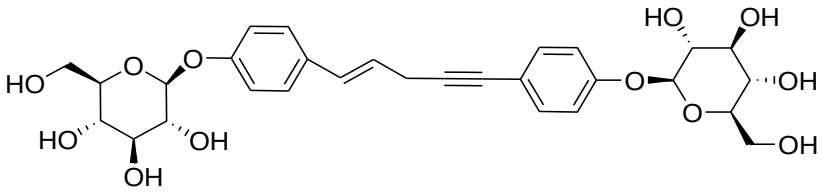
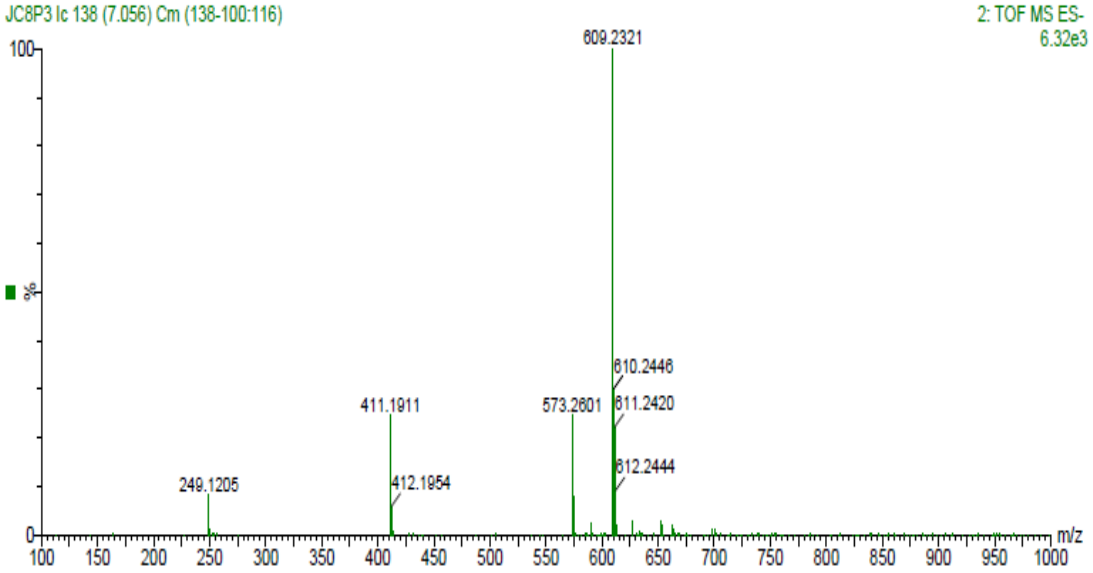
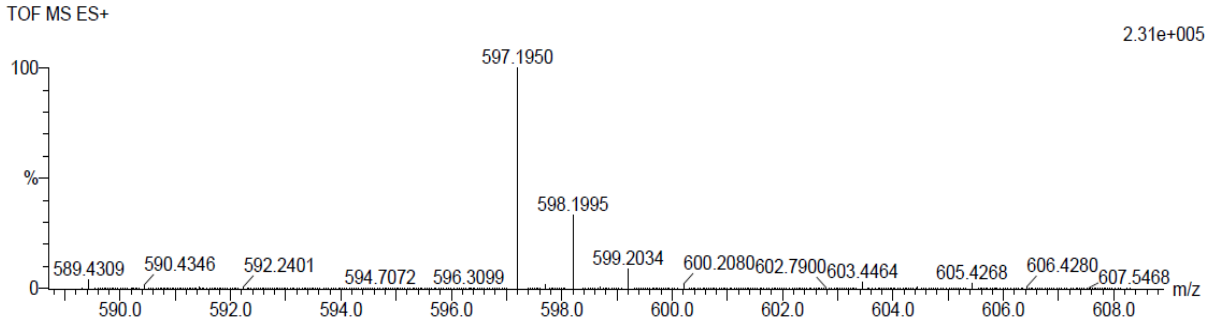


Plate 3h: High-resolution mass spectrum of bis-dehydroxyhypoxoside (3.16).







Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
597.1950	597.1948	0.2	0.3	12.5	471.4	0.0	C29 H34 O12 Na

Plate 4a: ^{1}H NMR spectrum of bisphenol A diglycidyl ether (3.17) in $CDCl_3$.

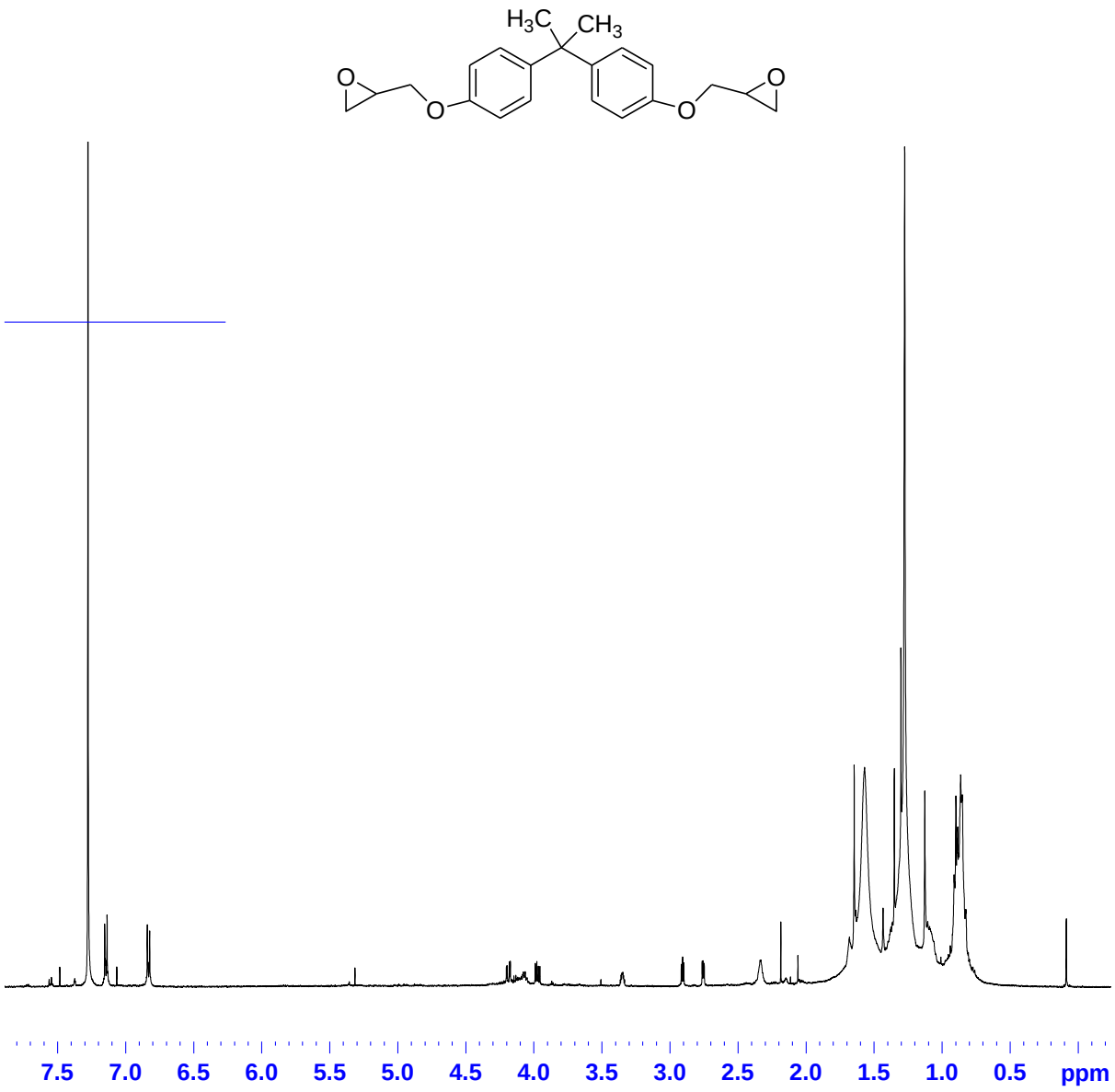


Plate 4b: COSY NMR spectrum of bisphenol A diglycidyl ether (3.17) in $CDCl_3$.

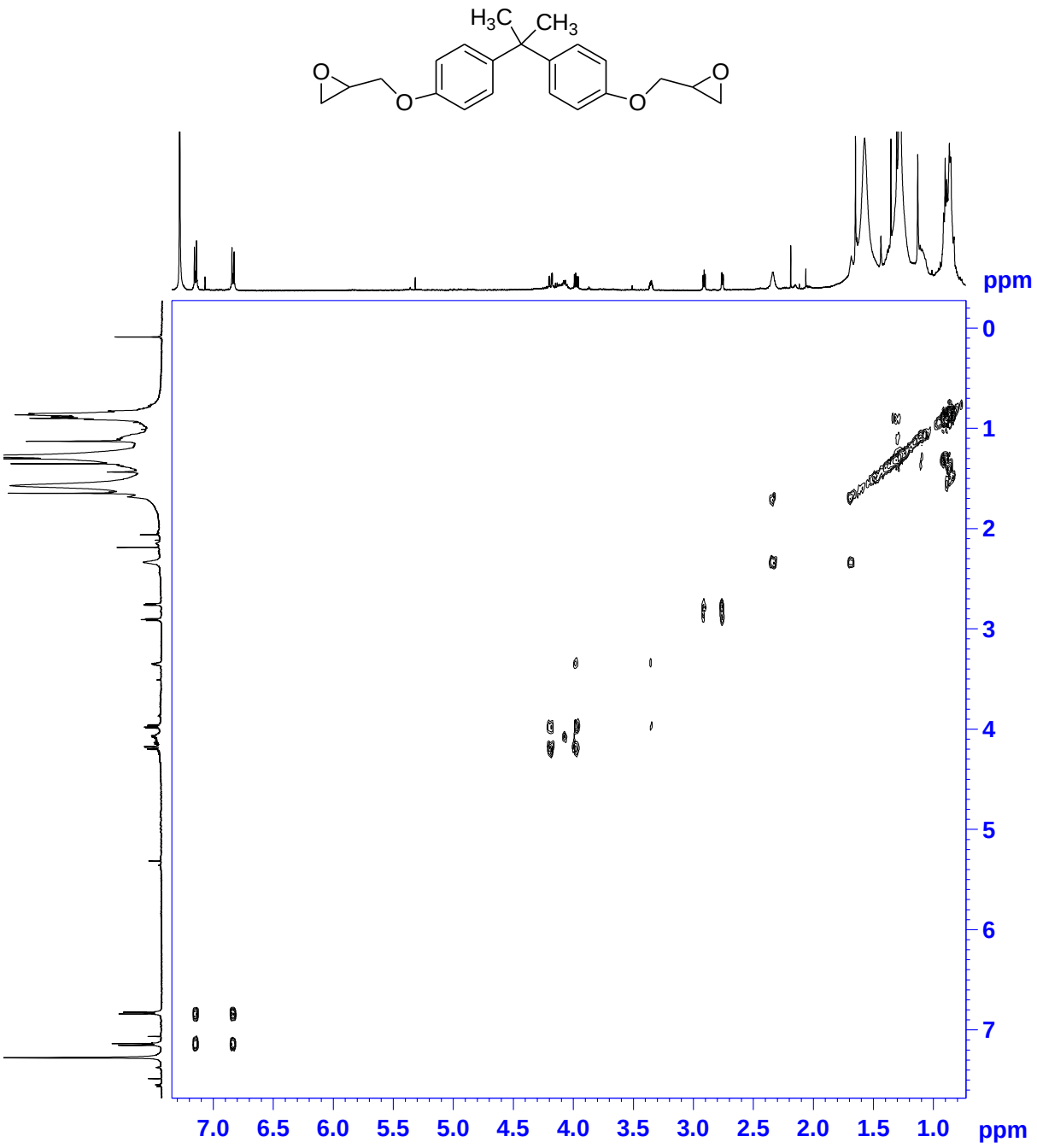


Plate 4c: ^{13}C NMR spectrum of bisphenol A diglycidyl ether (3.17) in $CDCl_3$.

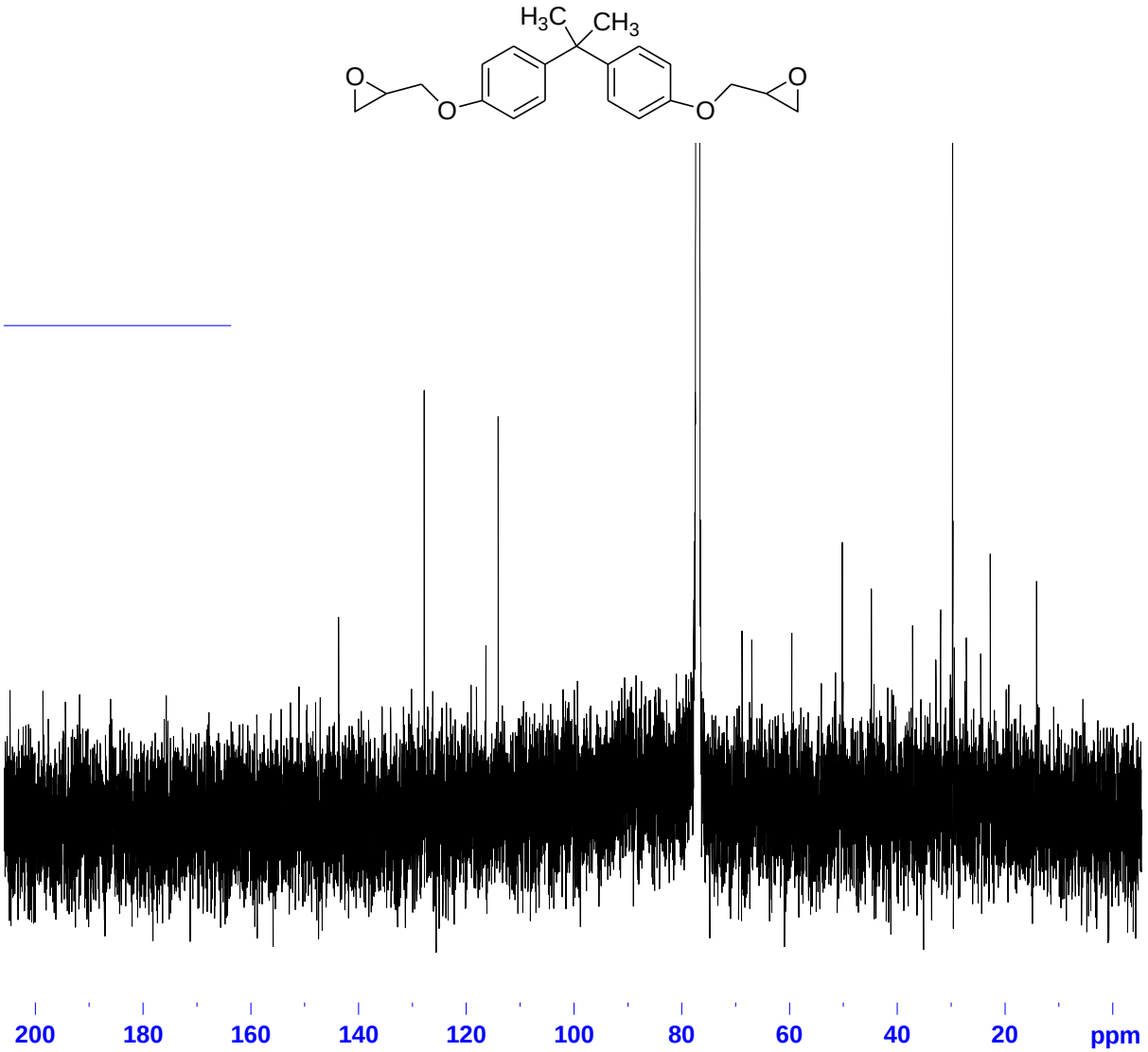


Plate 4d: HSQC NMR spectrum of bisphenol A diglycidyl ether (3.17) in $CDCl_3$.

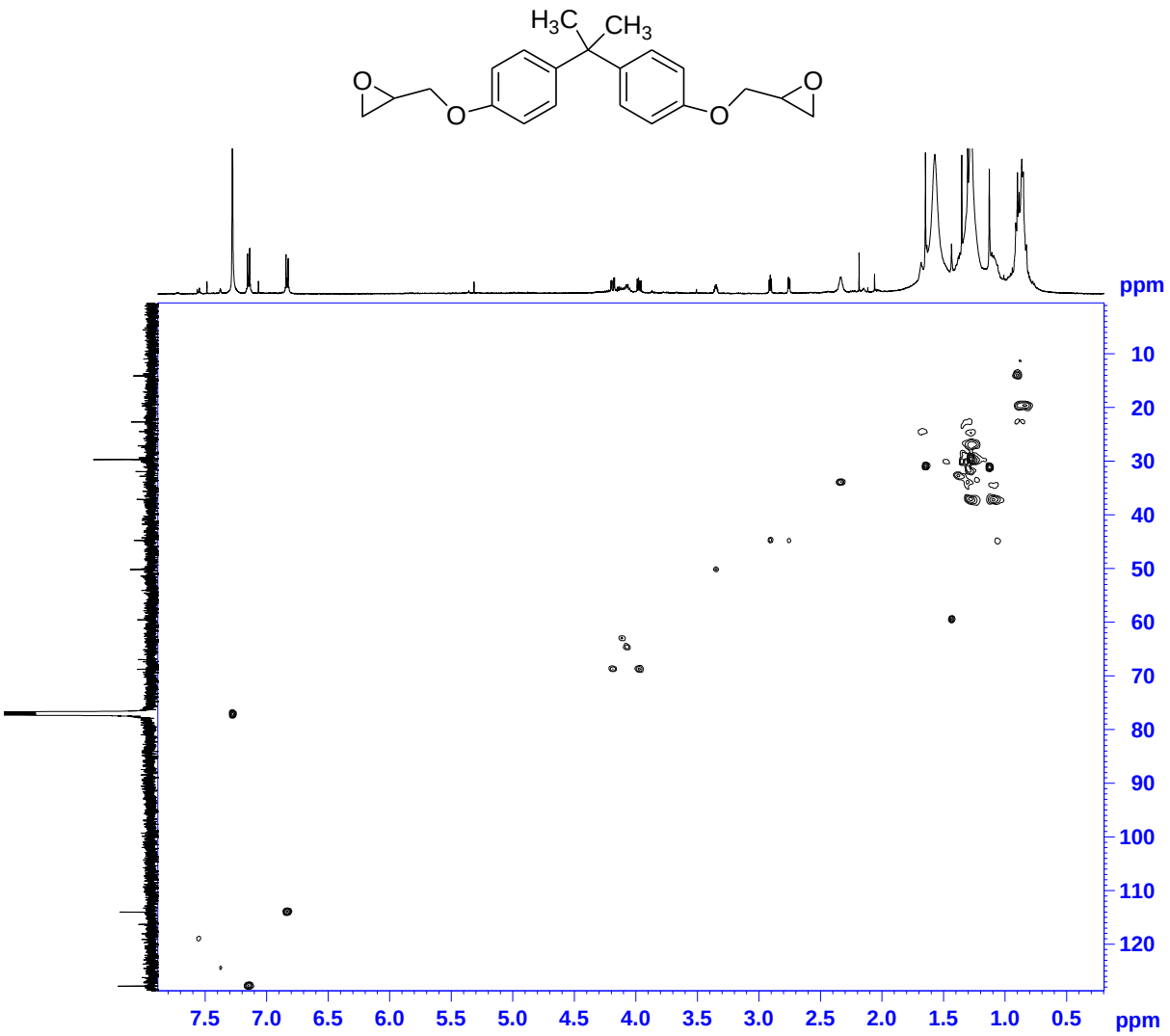


Plate 4e: HMBC NMR spectrum of bisphenol A diglycidyl ether (3.17) in $CDCl_3$.

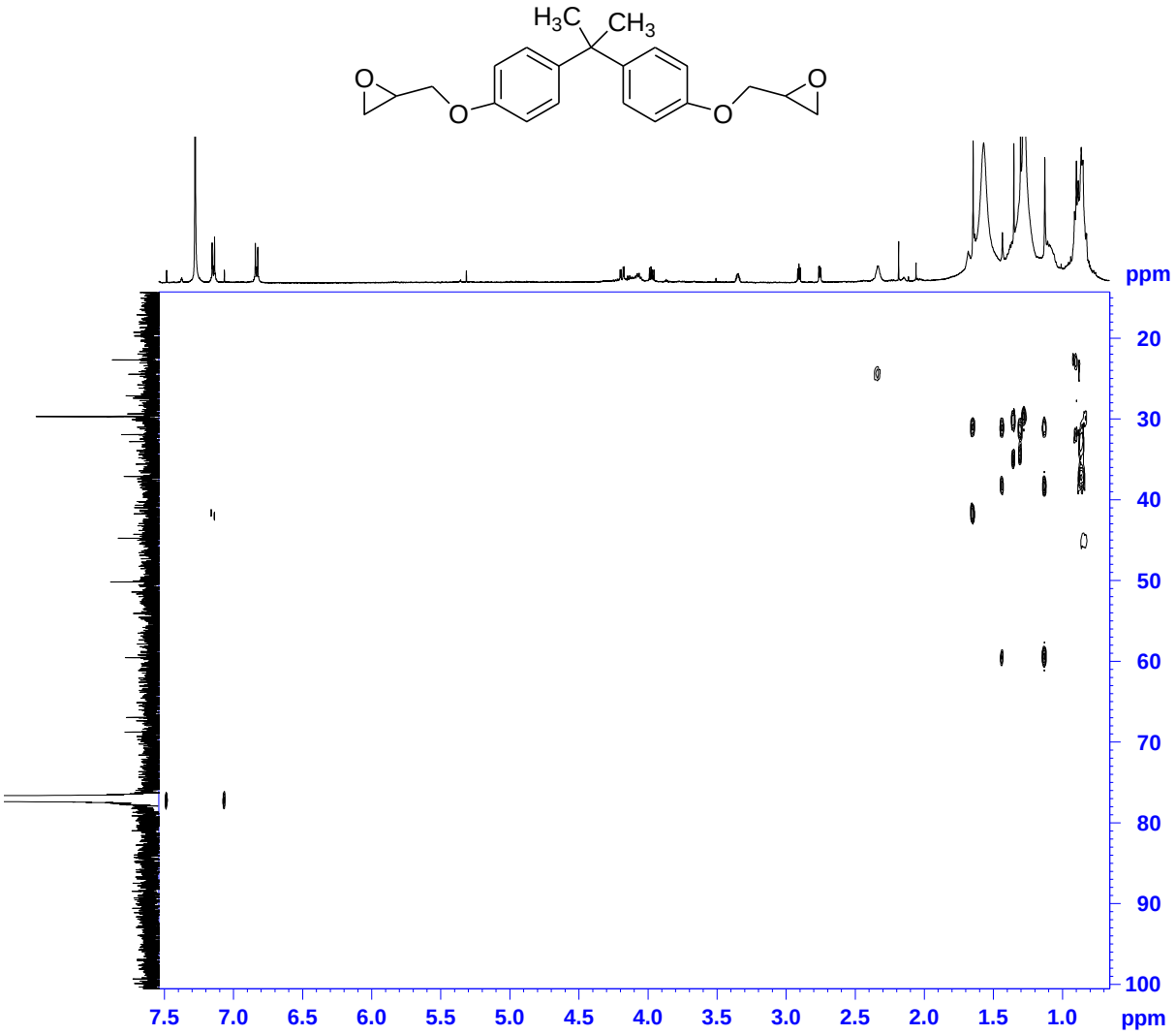
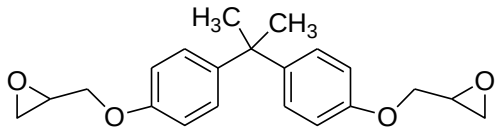


Plate 4f: High-resolution mass spectrum bisphenol A diglycidyl (3.17).



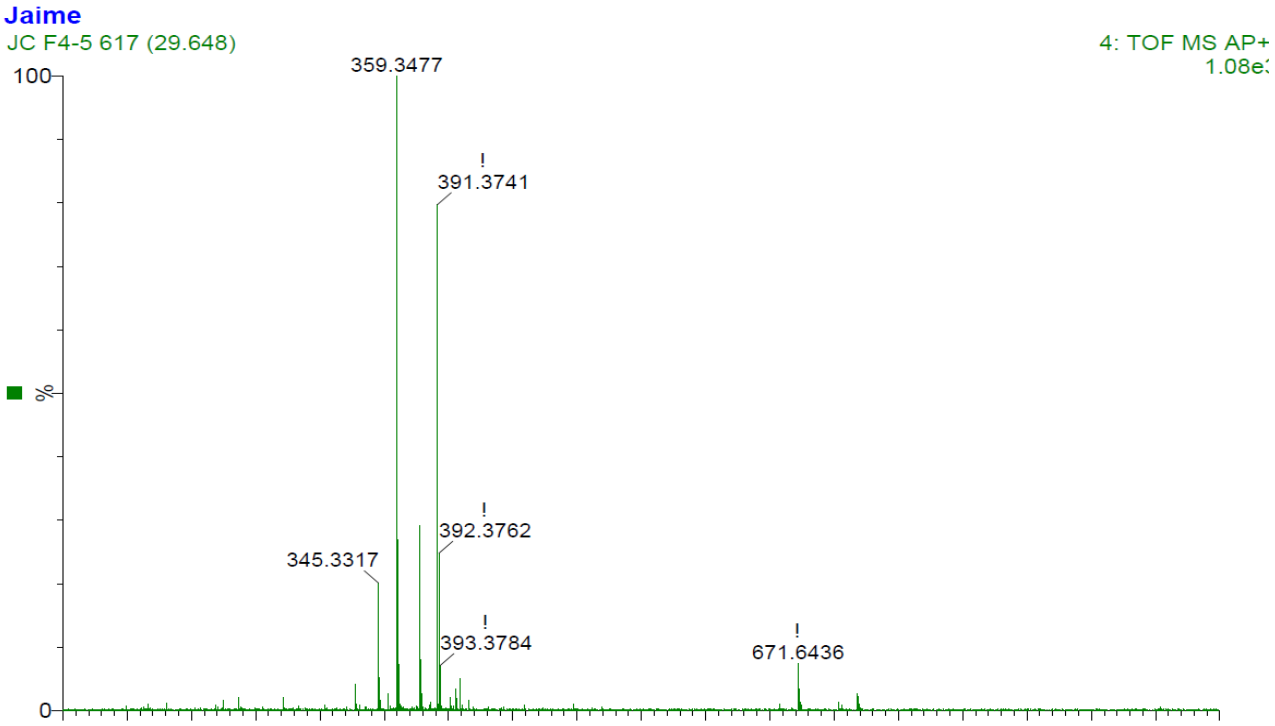


Plate 5a: **^{1}H NMR spectrum of sericic acid (4.29) in CD_3OD**.

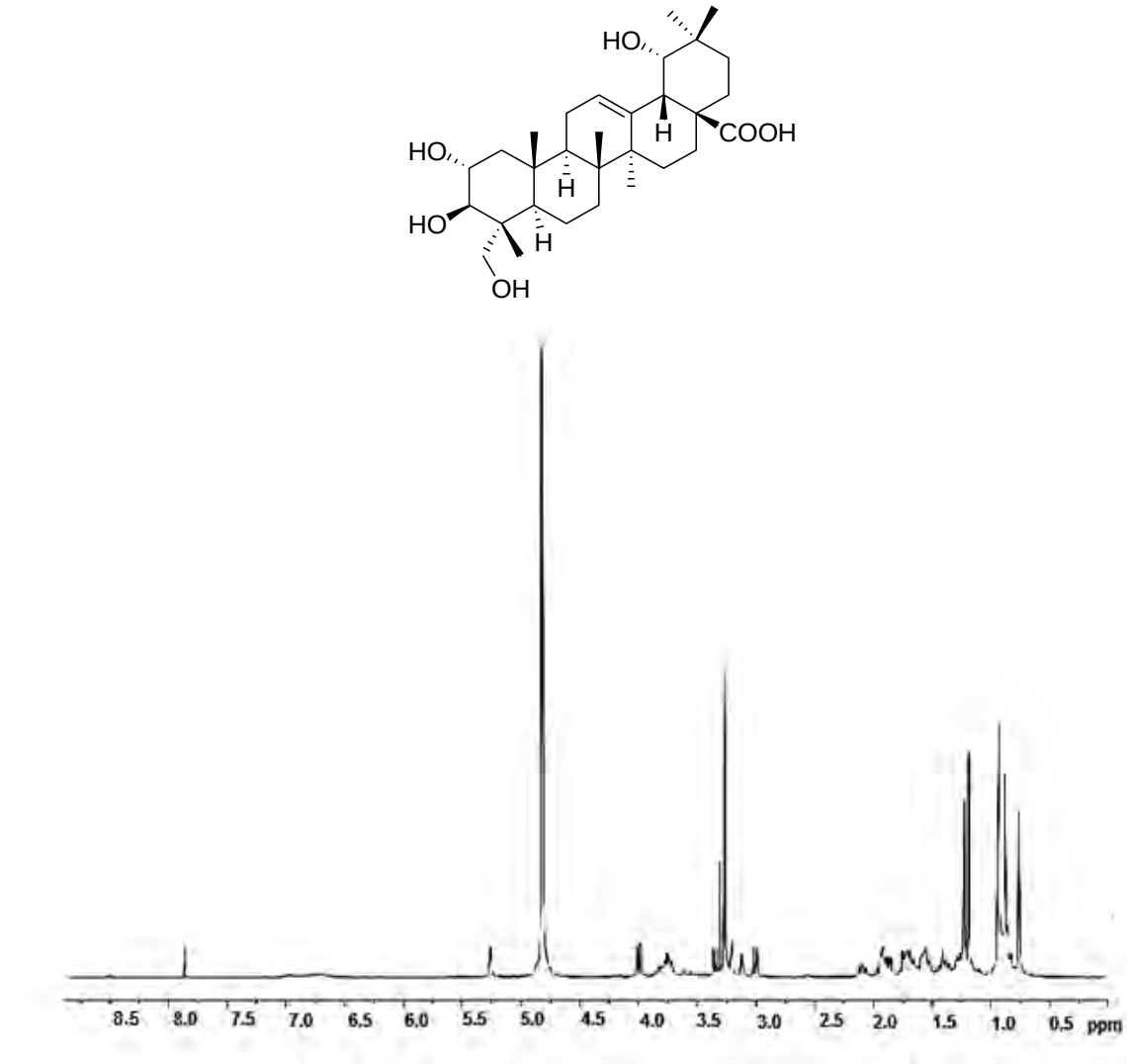
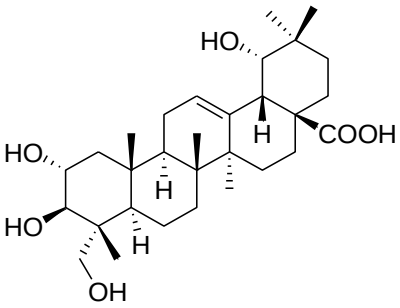


Plate 5b: ^{13}C NMR spectrum of sericic acid (4.29) in CD_3OD.



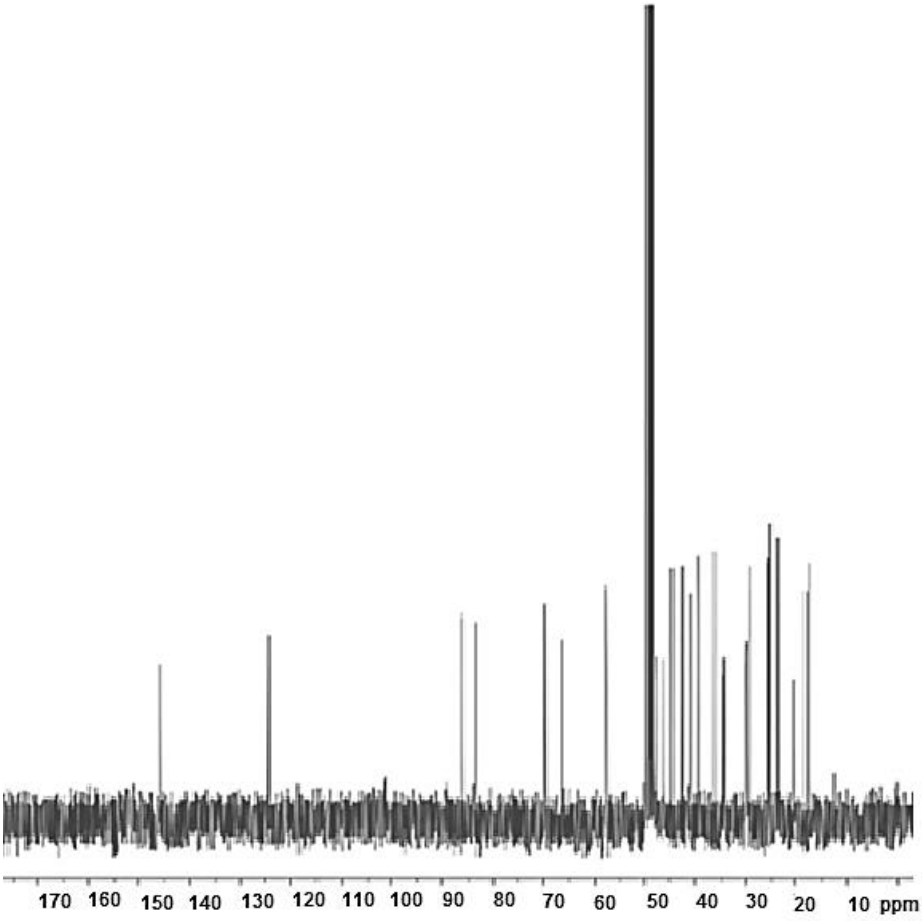


Plate 5c: HSQC NMR spectrum of sericic acid (4.29) in CD_3OD.

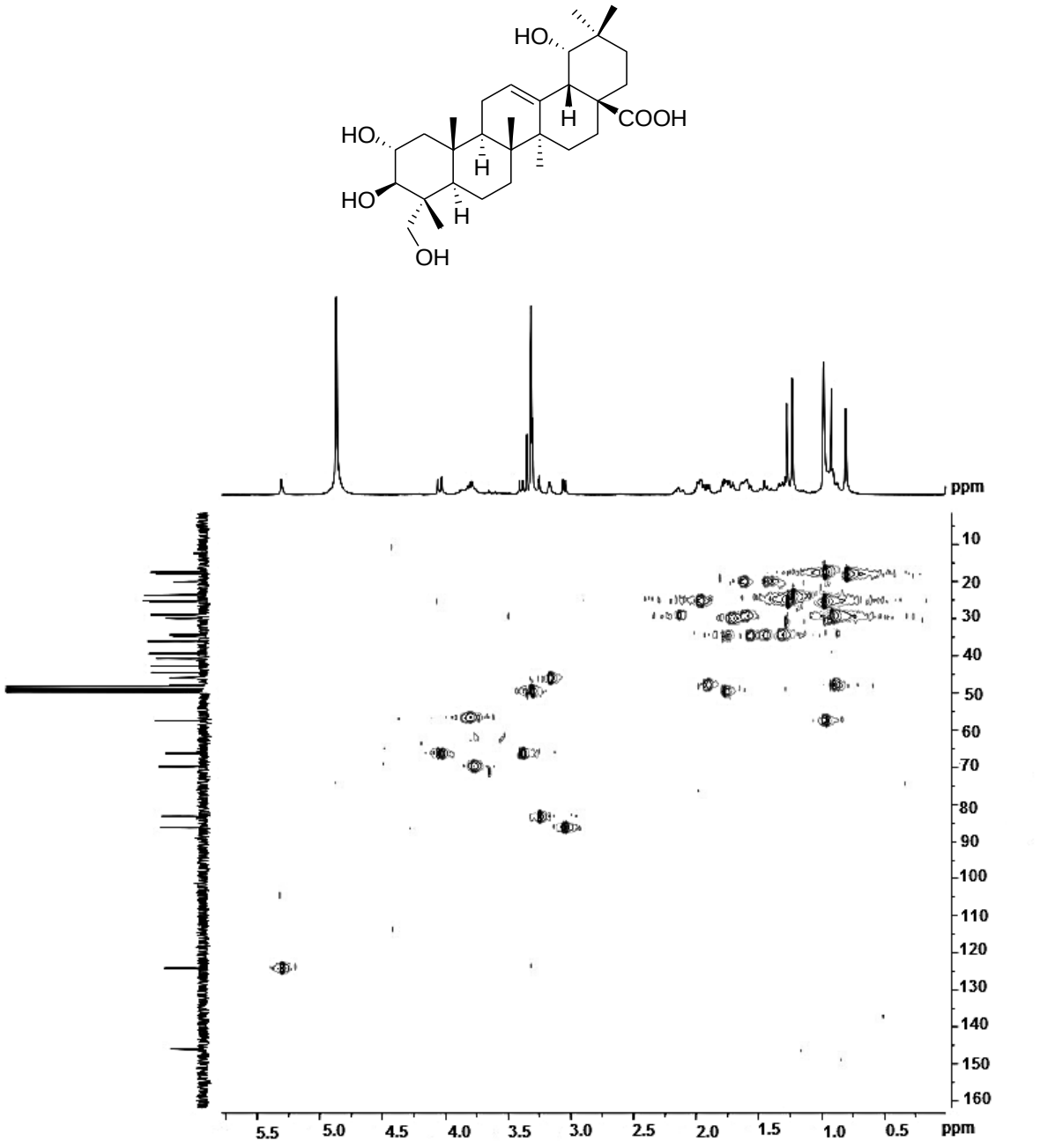


Plate 5d: HMBC NMR spectrum of sericic acid (4.29) in CD_3OD.

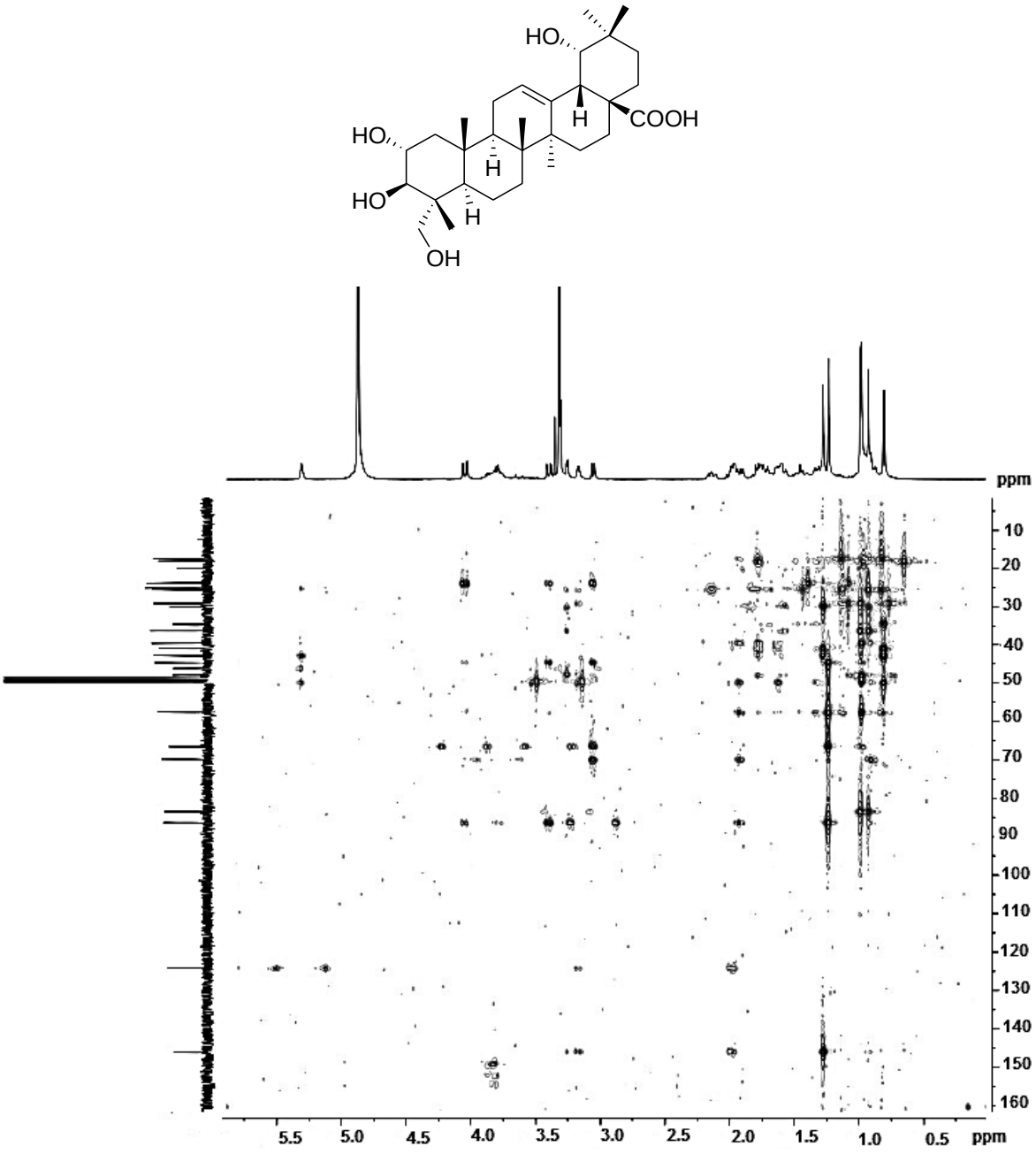
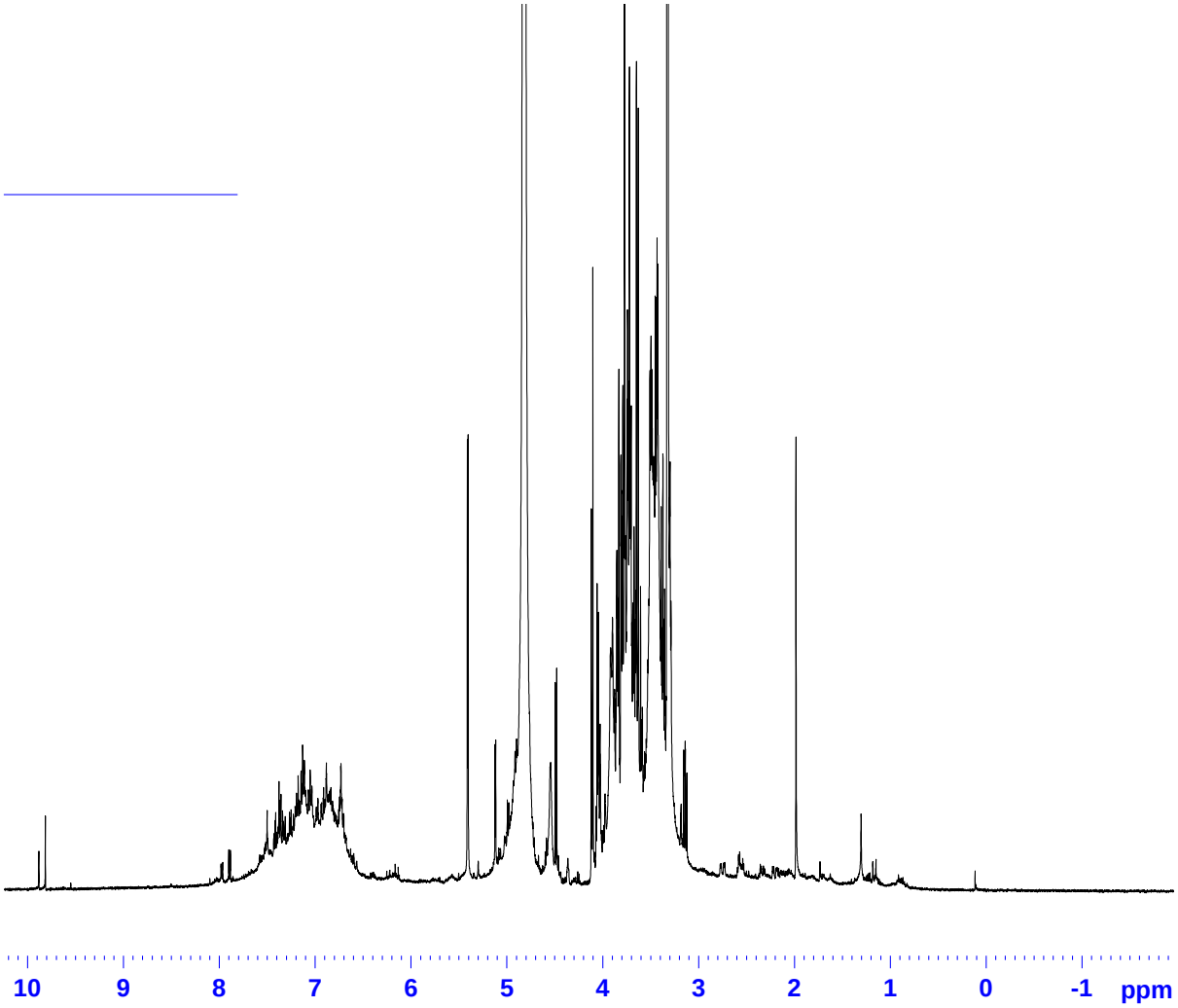


Plate 6a: ^{1}H NMR spectrum of the fraction one (F1) in CD_3OD



Publications and Conference presentations resulting from this thesis

Publications

Article in preparation

Dehydroxyhypoxoside B new compound from *Hypoxis colchifolia* Bak. Cumbe, J. T, van Heerden, F. R

Conference presentations

41st SACI Convention in East London (2013) Walter Sisulu University (poster presentation), Cumbe, J. T, van Heerden, F. R, Musabayane, C. T.
Identification of Hypoxoside in *Hypoxis colchicifolia* and *Hypoxis hemerocallidea* and Antidiabetic Activity of the Methanol extract of *Hypoxis colchicifolia*.

SACI - ACS Bi-national Organic Chemistry Conference (2014), thirteenth Frank Warren Conference, University of Stellenbosch (poster and flash presentation), Cumbe, J. T, van Heerden, F. R, Musabayane, C. T.
Antidiabetic Activity of two compounds from *Hypoxis colchicifolia*.