Addis Ababa University
Science Faculty
Chemistry Department
Organic Stream
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Phytochemical Investigation of the Pods of *Senna occidentalis*

Fekade Beshah

Advisor: Dr. Gizachew Alemayehu(PhD)

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A graduate project submitted to the Department of Chemistry, Science Faculty, AAU

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Abstract

Two anthraquinones: physion**(6)** and emodin**(7)** are isolated from the chloroform extract of Senna occidentalis pod. It also afforded one bianthraquinone: 5,7′- biphysion and anthraquinone derivative: trihydroxy-methoxy-methylanthraquinone, which are partially characterized. Several trace anthraquinones were also isolated. The compounds are identified on the basis of their color reactions and spectroscopic data.

1. Introduction

> "*If we wish to catch up with nature, we shall need to use the same methods as she does, and I can foresee a time in which physiological chemistry will not only make greater use of natural enzymes but also actually resort to creating synthetic ones*." - Emil Fisher

Natural product chemistry has originated from mankind's curiosity about color, taste, odor and cures for animals and plant diseases. A natural product is a chemical compound or substance derived from plants, microorganisms, or animals, which are fine biochemical factories for the biosynthesis of both primary and secondary metabolites [1]. It is just over hundred years, since Emil Fisher announced (1891) his elucidation of the structure of glucose. The complex alkaloids Vinblastin and Vincristine, which are highly antitumor agents, only succumbed to structure elucidation in 1964 [2].

Natural products are everywhere in our every day life. Some are active constituents of many medicines, vitamins, food additives, flavour and fragrances, agrochemicals and pesticides used for plant protection. The essential components of our life itself include proteins, carbohydrates, lipids, nucleic acids, vitamins, hormones, steroids, which are again Natural products. Natural pigments, dyes, alkaloids are also the commonly known natural products [1].

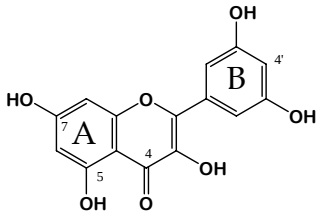
Natural products may be extracted from tissues of terrestrial plants, marine organisms or microorganism fermentation broths[3]. A crude (untreated) extract from any one of these sources typically contains novel, structurally diverse chemical compounds, which the natural environment is a rich source. A compound which can be prepared by total synthesis can be considered as natural product. Not all natural products can be fully synthesized and many natural products have very complex structures that are too difficult and expensive to synthesize on an industrial scale. These include drugs such as penicillin, morphine, and paclitaxel (Taxol) [1,3]. Such compounds can only be

harvested from their natural source - a process which can be tedious, time consuming, and expensive, as well as being wasteful on the natural resource. Semi synthetic procedures can sometimes get round these problems. This often involves harvesting a biosynthetic intermediate from the natural source, rather than the final (lead) compound itself. The intermediate could then be converted to the final product by conventional synthesis. This approach can have two advantages. First, the intermediate may be more easily extracted in higher yield than the final product itself. Second, it may allow the possibility of synthesizing analogues of the final product. The semisynthetic penicillins are an example of this approach [1].

Usually the compounds are identified as products of primary metabolism i.e. carbohydrates, nucleosides, amino acids and polymers derived from them or as products of secondary metabolism, i.e. phenolics, terpenoids, steroids and alkaloids. Primary metabolites are essentially ubiquitous and certainly essential for life, whilst the secondary metabolites are of restricted occurrence and of no apparent utility [2].

The drug discovery proccess increasingly requires the availability of larger number of compounds. Chemodiversity in nature offers a valuable source; for example secondary metabolites, previously regarded as waste products are now recognized for their resistant activity against pests and disease [4]. Secondary metabolites play ecologically significant roles in how the living organisms deal with their surroundings and therefore are important for their ultimate survival. The original definition of chemical compounds as ‘secondary ‘emerged from the notion that they are not absolutely essential to the survival and reproduction of plants [5].

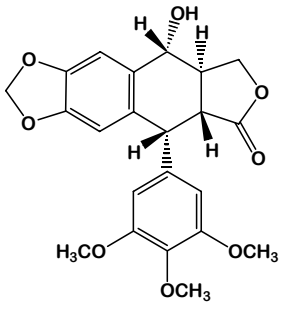
Phenolics are compounds with a parent compound phenol and a wide range of organic substances which are aromatic with hydroxyl substituents. The commonly occurring falvonol quercetin for example has five hydroxyl groups. While a small number of phenolics occur in animals, most are of plant origin. Indeed, the presence of a ‘phenolic fraction’ is a feature of all plants tissues. Among plant polyphenols, of which over 8000 are known, the falvonoides such as quercetin form the largest group [2].



(Structure of falvonol quercetin)

Phenolic quinones, lignans, xanthones, cumarins and other classes exist in considerable numbers.

Plant phenols are economically important because they make major contributions to the taste, flavour, and color of our food and drink. The flavour and taste of tea is related to the fact that the tea leaf contains up to 30 percent of its dry weight as polyphenol. Like wise the bitterness of beer is due to the content of the phlorogucinol derivative, humulone, while the red color of wine is imparted by anthocyanins such as the pigment malvin. In nature phenolics have significance in protecting plants from being over eaten by herbivores. They also act as chemical signals in the flowering and pollination of plants and in the process of plant symbioses (for example in nitrogen fixation) and plant parasitism [2]. Phenols are conveniently classified accordingly to the number of C-atoms in the basic skeleton (Table 1) Ranging from the simple phenols to the dimeric phenyl propanoids known as lignans(podophylloxin) [2].



(Structure of lignans (podophylloxin)

Phenolic compounds are found through out the plant kingdom, but the type of compounds present varies according to which plant group is under consideration [2].
Anthraquinone possess the anthracene skeleton and are represented as 9,10-oxo-anthracenes. They occur both in free and as glycosides in a large number of families including leguminoseae. Natural anthraquinones are in use both as natural dyes and drugs since time of immemorial. The age old dye alizarine is the hydrolysis product of the glycoside ruberythric acid.

Table 1. The major classes of phenolics in plants [2].

No.of C- atom	Basic Skeleton	Class	Examples
6	C_6	Simple phenols Benzoquinones	Catechol, Hydroquinone 2,6-Dimethoxybenzoquinone
7	C_6-C_1	Phenolic acid	p-Hydroxybenzoic , Salicylic
8	C_6-C_2	Acetophenones Phenylacetic acid	3-Acetyl-6-methoxybezaldehyde p-Hydroxyphenylacetic
9	C_6-C_3	Hydroxy cinamic acids Phenylpropenes Cumarines Isocumarines Chromones	Caffeic, ferulic Myristicin, eugenol Umbelliferone, aesculetin Bergenin Eugenin
10	C_6-C_4	Naphtaquinones	Juglone, Plumbagin
13	C_6-C_1- C_6	Xanthones	Mangiferin
14	C_6-C_2- C_6	Stilbenes Anthraquinones	Lunularic acid Emodin
15	C_6-C_3- C_6	Falvonoides Isofalvonoides	Quercetin, Malvin Genistein
18	$(C_6$-$C_3)_2$	Lignans	Podophyllotoxin
20	$(C_6$-C_3-$C_6)_2$	Biflavonoides	Amentoflavone
n	$(C_6$-$C_3)_n$ $(C_6)_n$ $(C_6$-C_3-$C_6)_n$	Lignins Catechol melanins Flavolons	- - - (Condensed tannins)

Similarly many anthraquinone derivatives have been in use as purgatives/laxatives. These anthraquinine derivatives contain hydroxyl, hydroxymethyl, methyl and carboxyl groups [1].

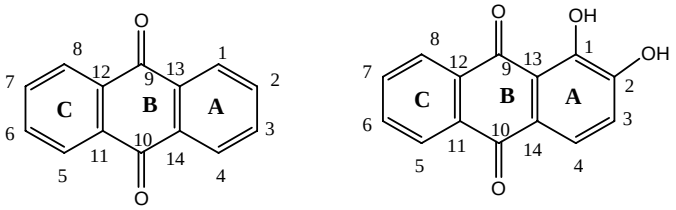


Figure 1. Anthraquinone skeleton and alizarin

Historically alizarine is one of the oldest commercially valuable red dyes which was isolated as back as 1826 from madder root, *Rubia tinctoria*[1].

Many higher plants contain novel metabolites with antimicrobial and antiviral properties. Pharmacognosy provides the tools to identify select and process natural

products destined for medicinal use. Usually, the natural product compound has some form of biological activity and that compound is known as the active principle - such a structure can act as a lead compound (not to be confused with compounds containing the element lead). Many of today's medicines are obtained *directly* from a natural source. On the other hand, some medicines are developed from a lead compound originally obtained from a natural source. This means the lead compound:

- can be produced by total synthesis, or
- can be a starting point (precursor) for a semisynthetic compound, or
- can act as a template for a structurally different total synthetic compound.

Plants have always been a rich source of lead compounds (e.g. morphine, cocaine, digitalis, quinine, tubocurarine, nicotine, and muscarine)[6]. Many of these lead compounds are useful drugs in themselves (e.g. morphine and quinine), and others have been the basis for synthetic drugs (e.g. local anaesthetics developed from cocaine). Clinically useful drugs which have been isolated from plants include the anticancer agent paclitaxel (Taxol) from the yew tree[1], and the antimalarial agent artemisinin from *Artemisia annua* [7]. Plants provide a large bank of rich, complex and highly varied structures which are unlikely to be synthesized in laboratories.

Microorganisms such as bacteria and fungi have been invaluable for discovering drugs and lead compounds. These microorganisms produce a large variety of antimicrobial agents which have evolved to give their hosts an advantage over their competitors in the microbiological world. The screening of microorganisms became highly popular after the discovery of penicillin [1].

With the advent of improved chromatographic separation techniques, the separation of various natural products including positional and stereoisomer, is achieved routinely. Newer spectroscopic techniques such as two dimensional high resolution nuclear magnetic resonance spectroscopy, Infrared and Raman spectroscopy, X-ay crystallography, high resolution electron microscopy and mass spectrometry have simplified the structural elucidation of new natural products [1].

2. *Senna occidentalis* And Its Medicnal Uses

A medicinal plant is one whose one or more of its organs contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs [8]. Herbal medicine, which is the use of medicinal plants or drugs from medicinal plants in the treatment and cure of sickness and diseased conditions, has been with man since the beginning of time.

Botanical backgrounds

The family Leguminosae comprises some 748 genera and 19700 species widely distributed over most of the world. This big family is divided in to 3 sub families: mimosoideae, caesalpinoideae and pappilionoideae. The genus senna which belongs to the leguminosae family and caesalpinoideae subfamily has about 240 species distributed throughout the tropics and subtropics [9]. *Senna occidentalis* is introduced from South America. There are 38 species in Africa. In Ethiopia there are eighteen species belonging to the genus [10]. They are namely (Synonyms are indicated in brackets): *Senna petersiana(Cassia petersiana), Senna septemtrionalis (Cassia septemtrionalis, Cassia laevigata, Cassia floribunda), Senna singueana (Cassia singueana, Cassia sabak, Cassia goroatensis), Senna baccarinii(Cassia baccarinii), Senna multiglandulosa (Cassia multiglandulosa, Cassia tomentosa), Senna bicapsularis (Cassia bicapsularis), Senna occidentalis (Cassia occidentalis), Senna sophera (cassia sophera), Senna obtusifolia(Cassia obtusifolia, Cassia tora), Senna siamea (Cassia siamea), Senna didymobotrya (Cassia didymobotrya), Senna ruspolii (Cassia ruspolii), Senna longiraceemosa(Cassia ongiraceemosa), Senna ellisiae (Cassia ellisiae), Senna truncata (Cassia truncata), Senna italica (Cassia italica), Senna holosericea (Cassia holosericea), Senna allexanderina (Cassia allexanderina, Cassia angustifolia*)[9,10].

Medicinal Application

Several species of senna have important medicinal properties and are used in both traditional and modern medicine [11, 12]. As the other species *S. occidentalis* has many uses. The roasted seeds can be used as a coffee substitute [9], and used against stomach

disorders and rheumatism [12]. *Cassia occidentalis* is used as medicinal plant and traditional medicine in different part of the world. The plant is efficacious in curing dodhis itch, ring worm and other skin diseases. It is also a plant with hypoglycaemic property. The plant has many other therapeutic properties, including roots against syphilis. As with many Senna species, the plant has been established as a purgative [12,13], insecticide [14] and potentially toxic to livestock [15].

The seed is bitter, has purgative properties, and is used as a general tonic for weakness. The root is applied externally for rheumatism [12]. It is also used as a diuritic, liver detoxifier, as a hepatotonic (balances and strengthens the liver). Further, used in whooping cough and convulsion [16,17].

Pharmacological studies

The plant has been used as natural medicine in many tropical areas. Its roots, leaves, flowers, and seeds have been employed in herbal medicine. *C. occidentalis* leaves ethanol extract has anti-inflammatory activity at a dose of 2000 mg/kg. It may exert its anti-inflammatory activity by inhibition of phospholipase A2, resulting in the reduced availability of arachidonic acid, a precursor of prostaglandin biosynthesis, by stabilization of the lysosomal membrane system [14,18]. *C. occidentalis* exhibited anti-dermatophyte activity [19,20]. *C. occidentalis* showed antibacterial activity against Salmonella typhi [21,22]; and against Bacillus subtilis and Staphylococcus aureus [37].

The hepatoprotective effect of aqueous-ethanolic extract of leaves of *C. occidentalis* was studied on rat liver damage induced by paracetamol and ethyl alcohol by monitoring serum transaminase (aspartate amino transferase and serum alanine amino transferase), alkaline posphatase, serum cholesterol, serum total lipids and histopathological alterations. The extract of leaves of the plant produced significant hepatoprotection [23].

The antimalarial activity of *C. occidentalis* crude extracts was confirmed. The plant showed more than 60% inhibition of the parasite growth in *vitro* at a test concentration of 6μg/ml [24,25]. The number of hydroxyl groups in the anthraquinones seemed to play an important role in the degree of cells growth inhibition. Anthraquinones with

two or three hydroxyl groups were more effective than those with no hydroxyl groups [14]. The aqueous extract of *C. occidentalis* at the dose of 100 mg/kg for 14 days modulated hepatic drug metabolizing enzymes. It is suggested that by a similar mechanism, it may be influencing the hematotoxic and immunotoxic responses of cyclophosphamide[26].

The *cassia occidentalis* poisoning in children seems to affect mainly three systems: hepatic, skeletal muscles and brain. The exact toxic principles are yet to be identified but various anthraquinones and their derivatives are usually blamed for its toxicity [27].

Besides its medical application, the seed of *Senna occidentalis* is a rich source of galactomannan gum. The gum derived from the seed endosperm can be potentially used in a number of industries to replace the conventional gum with a view to utilize the gum for broader applications [28].

3. Secondary Metabolites from *Senna occidentalis*

Cassia plants are well known for a group of chemicals with strong laxative actions, called anthraquinones and a bianthraquinone [13,14,29,30]. An interaction between rehien anthrone, the active metabolites of sennosides and the immune cells of the colon is suggested as a base for laxative activity [14]. Chysophanol and emodin, both free and their glycosides and free physcion were found in the leaves, both free and as glycosides of rhein and aloe emodin in the roots, and both free and glycosides of chrysophanol and physcion in the seeds of *C. occidentalis*.

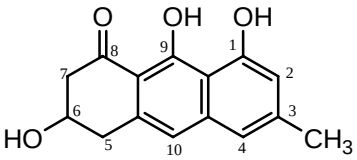
Two bis (tetrahydro) anthracene derivatives, occidentalol-1 **(28)** and occidentalol-II **(29)** were isolated from the roots of *C. Occidentalis* alongue with chrysophanol **(3)**, emodin**(7)**, questin**(13),** germichrysone**(1)**, methylgermitorosone**(4)** and singueanol-I**(30)**. These are dimers of preanthraquinones. The structures were established on the basis of spectral evidence [31]. The commonly known groups of anthraquinones isolated from *Senna occidentalis* and the parts of plants considered are summarized bellow in tables 2 to 5.

3.1 Preanthraquinones From *Senna occidentalis*

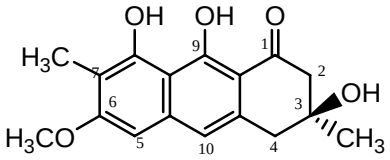
Preanthraquinones are the biosynthetic precursors to the corresponding anthraquionones. Some of the most common representatives for this group are listed below on table 2.

Table 2. Preanthraquinones

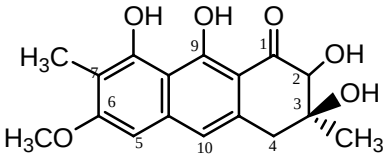
No.	Name	Plant part	Reference
1	Germichrysone	Seed, Root	31,32
2	7-methyltorosachrysone	Tissue culture	31,32
3	Germitorosone	Tissue culture	32
4	Germitorosone-9-methyl ether	Tissue culture	32



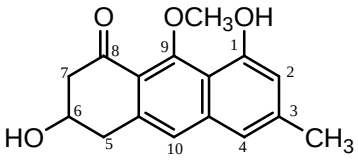
(1) Germichrysone



(2) 7-methyl torosachrysone



(3) Germitorosone



(4) Germitorosone-9-methyl ether

Figure 2. Structures of preanthraquinones

3.2 Anthraquinones From *Senna occidentalis*

Anthraquionones are widely isolated and reported throughout the species. Those reported are listed below on table 3 with their respective references.

Table 3. Anthraquinones from *Senna occidentalis*

No.	Name	Plant part	Reference
5	Chrysophenol	Root , seed	31
6	Physcion	Leaves , seed	10
7	Emodin	Root , leaves	31,33
8	1,4,5-Trihydroxy-7-methoxy-3-methylanthraquinone	Root , leaves	34
9	7-methyl physcion	Seed	35,32
10	1,8-dihydroxy-2-methylanthraquinone	Aerial part	10
11	1,4,8-Trihydroxy-6-methoxy-2-methyl anthraquinone	Root	31
12	Fallacinol	Tissue culture	10
13	Questin	Root	31
14	Islandicin	Root	38
15	Alo-emodin	Tissue culture	10
16	Helminthosporin	Root	38
17	Xanthorin	Root	38
18	Rhein	Tissue culture	10
19	Torosachrysone	Seed	32
20	Physion -9 anthrone	Seed	32

21	1,4,6,8-Tetrahydroxy-2-mehtylanthraquinone	Seed	39
22	1,3,8-Trihydroxy-2,6-dimehtylanthraquinone	Tissue culture	32
23	Xantorin-5-mehtylether	Root	35

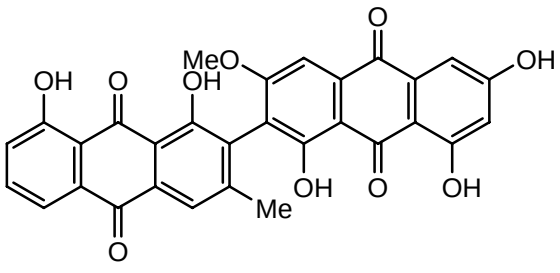
The structures of these anthraquinones are based on the parent structure of anthraquinone shown on figure 1.

3.3. Bianthraquinones From *Senna occidentalis*

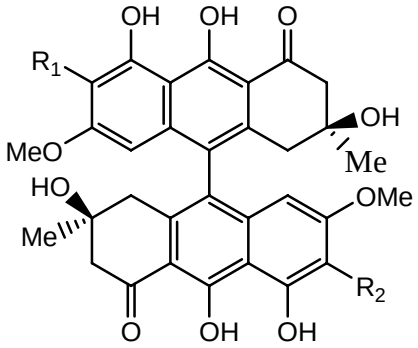
A number of dimeric anthraquinones have been isolated as C-C linkage between two units of anthraquinones. The known and reported are summarized in table 4.

Table 4. Bianthraquinones from *Senna occidentalis*

No.	Name	Plant part	Reference
24	Cassiamine A(2,2′-emodin-chrysophenol bianthrquionone	Leaves	35
25	Cassiamine C(2,2′-chrysophenol bianthrquionone	Leaves	35
26	Siameanin(4,4′-chrysophenol bianthrquionone	Leaves, roots	34,35
27	Palmidin D (Chrysophenol-physcion-10,10′ -bianthrquionone	Leaves, roots	34,35
28	Occidentalol I	Root	31
29	Occidentalol II	Root	31
30	Singueanol I	Root	31
31	Ararobinol(Chrysophanol-10,10-bianthrone)	Seed	32



(24) Cassiamin A



(30) Singueanol- I R_1=R_2= Me,
(28) Occidentalol- I R_1=Me,R_2= H
(29) Occidentalol- II R_1=R_2= H

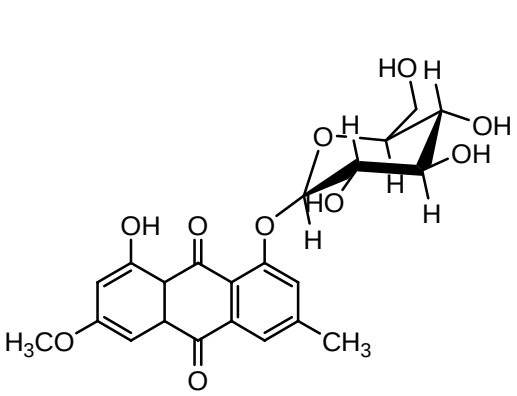
Figure 3. Representatives of bianthroaquinones

3.4. Glycosides From *Senna occidentalis*

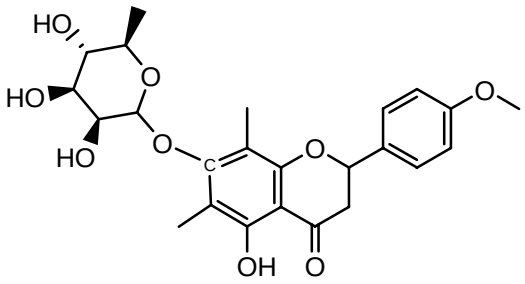
The presence of sugar moiety in anthraquinones and flavonoids and also the types of sugar though presumably has no direct activity of its own, is a paramount for the enhancement of pharmacological activity of the aglycone. As the sugar increases the solubility of the active aglycones so that they can pass via the blood stream to the site of action. Anthraquinones are prone to metabolic detoxification. The presence of a sugar group confers a resistance on the glycosides such that it withstands this detoxification of the body. Glycosides of *Senna occidentalis* are given below on Table 5.

Table 5. Glycosides from *Senna occidentalis*

No.	Name	Plant part	Reference
32	Physcion 1-O-β-D-glycopyranoside	seed	40
33	Matteucinol-7-rhamnoside	leaves	41
34	Jaceidin-7-rhamnoside	leaves	41
35	Jaceidin-7-O-Neophesperidoside(Ombuin) -3-digalctoside	Pod	10
36	Jaceidin-7-O-α-L-Rhamnopyranoside	Leaves	41



(32) Physcion 1-O-β-D-glycopyranoside



5,7-dihydroxy-4'-methoxy-6,8-dimethyl flavanone-7-rhamnoside

(33) (Matteucinol-7-rhamnoside)

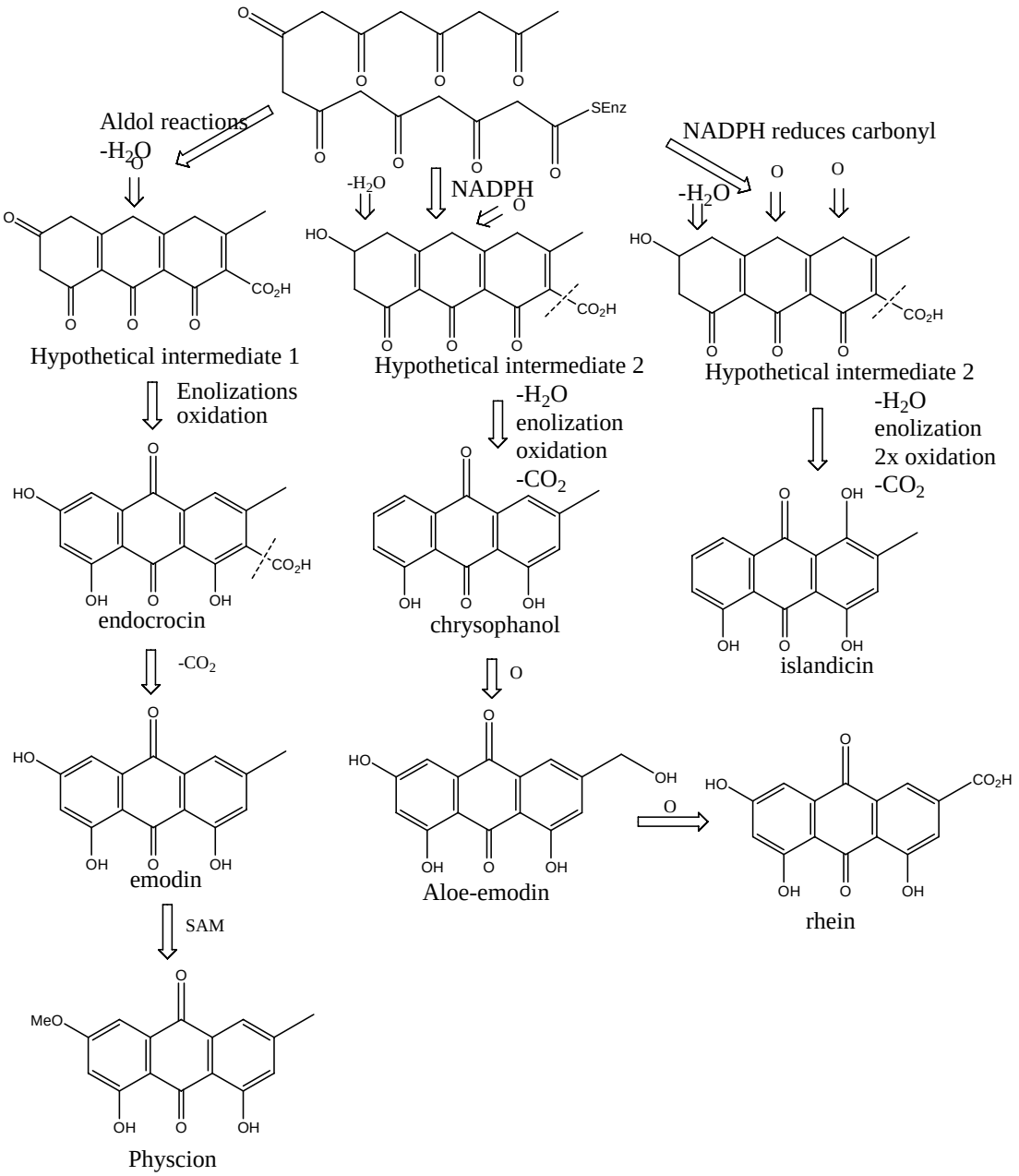
Figure 4. Representatives of glycosides

4. Biosynthesis of Anthraquinones

The synthesis of organic products *in vivo* is referred to as biogenesis or biosynthesis [1]. Natural quinones are derived from three principal intermediates: acetate, malonate and skikimate and two major pathways: Acetate-malonate(octaketide) pathway and Shikimic pathway. Anthraquinones are the largest groups of quinones and are widely spread in lower and higher plants and also in the animal kingdom. They are present as glycosides in young plants. Fungal anthraquinones and plant anthraquinones with hydroxyl groups are derived from polyketides where as plant anthraquinones devoid of hydroxyl groups in one ring e.g. alizarin came from mixed pathways [42].

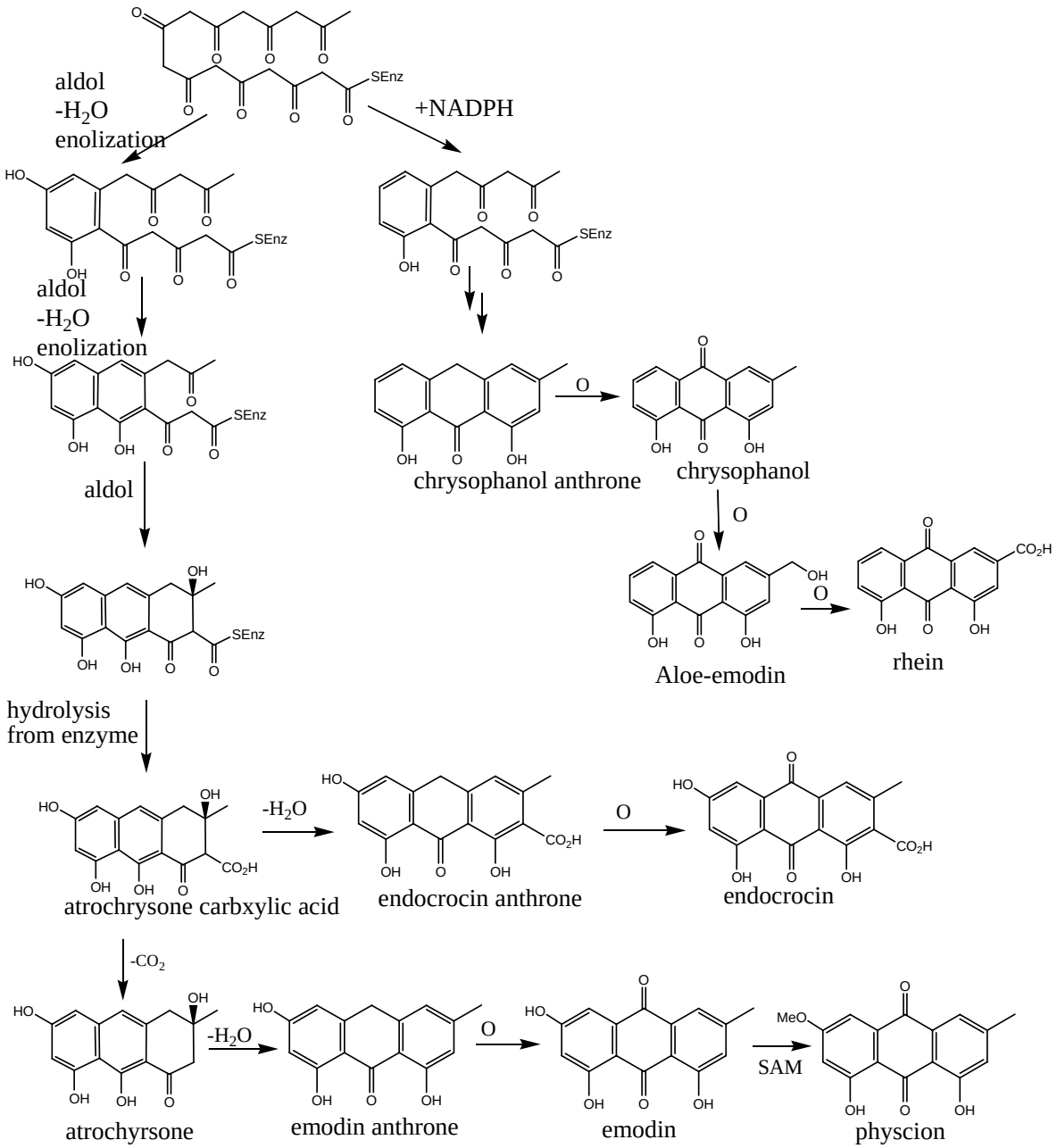
A number of natural anthraquinone derivatives are excellent examples of acetate-derived structures (octaketide pathway). They arise via different folding, O-methylation, side chain oxidation, nuclear hydroxylation, or elimination of hydroxyl groups, chlorination, dimerization via phenol oxidation etc[42]. Endocrocin (Scheme 1) is formed by folding a polyketide containing eight C_2 units to form the periphery of the carbon skeleton. Three aldol-type condensations would give a hypothetical intermediate 1, and, except for a crucial carbonyl oxygen in the centre ring, endocrocin results by enolization reactions. The additional carbonyl oxygen must be introduced at some stage during the biosynthesis by an oxidative process. Emodin, a metabolite of some *Penicillium* species, but also found in higher plants, e.g. *Rhamnus* and *Rumex* species, would appear to be formed from endocrocin by a simple decarboxylation reaction which is facilitated by the adjacent phenol function [43]. Shortly emodin is constructed from one acetate and seven malonate units [42].

O-Methylation of emodin would then lead to physcion. Islandicin is another anthraquinone pigment produced by *Penicillium islandicum*, and differs from emodin in two ways. One hydroxyl is missing, and a new hydroxyl has been incorporated adjacent to the methyl. The structure of intermediate 2 shows the result of three aldol condensations and reduction of a carbonyl. A dehydration reaction, two oxidations, and a decarboxylation are necessary to attain the islandicin structure.



Scheme 1. Acetate Pathway [43]

Chrysophanol, aloe-emodin, and rhein are interrelated by a sequential oxidation of the methyl in chrysophanol to a hydroxymethyl in aloe-emodin, and a carboxyl in rhein (scheme 1 and 2) [43]. The assembly of the anthraquinone skeleton is achieved in a step-wise sequence. After the polyketide chain is folded, the ring at the centre of the fold is formed first, followed in turn by the next two rings. The pathway outlined for the biosynthesis of endocrocin and emodin is shown in scheme 2. Mechanistically, there is little difference between this and the acetate pathway on scheme 1, except the sequence of reactions.



Scheme 2. Biosynthesis of endocrocin and emodin [43]

Decarboxylation appears to take place before aromatization of the last-formed ring system, and tetrahydroanthracene intermediates such as atrochrysone carboxylic acid and atrochrysone are involved. These dehydrate to the anthrones: endocrocin anthrone and emodin anthrone, respectively, prior to introduction of the extra carbonyl oxygen derived from O_2 as a last transformation in the production of anthraquinones [43]. Most anthraquinones of Leguminosae have substituents on both rings; therefore, the most plausible biogenetic route of them appears to be polyketide origin.

Emodin, physcion, chrysophanol, aloe-emodin, and rhein form the basis of a range of purgative anthraquinone derivatives found in long established laxatives such as Senna, Cascara, Frangula, Rhubarb, and Aloes. The free anthraquinones themselves have little therapeutic activity and need to be in the form of water-soluble glycosides to exert their action. Although simple anthraquinone O-glycosides are present in the drugs, the major purgative action arises from compounds such as cascarosides, e.g. cascaroside A, which are both O- and C-glycosides, and sennosides, e.g. sennoside A (Figure 5), which are dianthrone O-glycosides [43].

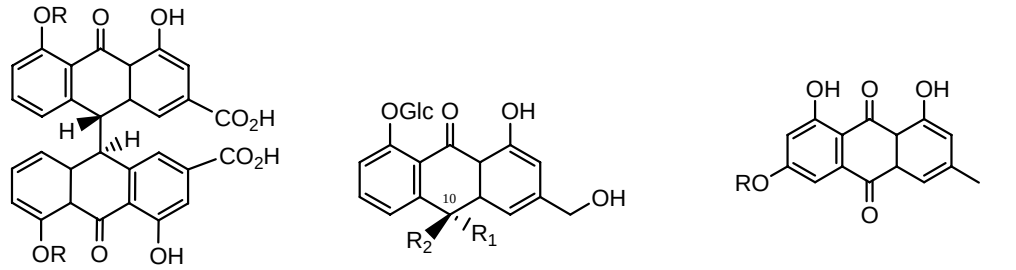
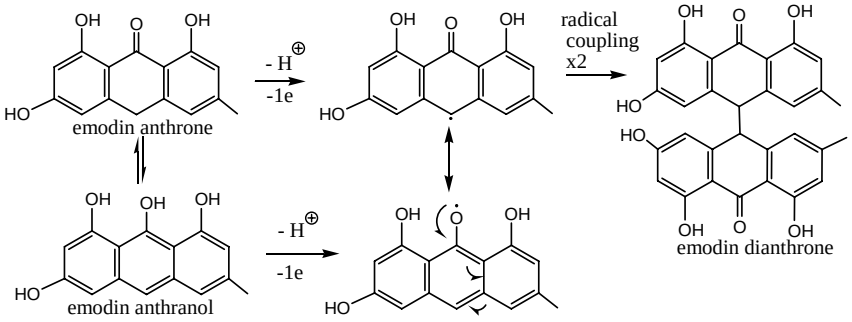


Figure 5. Sennosides , cascarosides and frangulins[43]

A one-electron oxidation allows oxidative coupling of two anthrone systems to give a dianthrone (Scheme 3). This can be formulated as direct oxidation at the benzylic $-CH_2-$, or via the anthranol, which is the phenolic tautomer of the anthrone (Scheme 3). Glycosylation of the dianthrone system would then give a sennoside-like product [43]. Bacterial degradation of sennosides produces rehin which is thought to be the active substance responsible for the cathartic effect of senna[44].



Scheme 3. One electron oxidation of emodin anthrone [43]

The reactions of scheme 3 can be achieved chemically by passing air into an alkaline solution of emodin anthrone [43].

5. Identification of Anthraquinones

5.1. Color reaction

Color reactions are useful particularly at the beginning of an investigation when crude extracts or even natural tissue may yield information of value. Very little material is required and the reaction may be carried, by spraying chromatograms. For hydroxyquinones, the color changes are more striking in alkaline solution and re-oxidation(by air) is more rapid. The characteristic colors given by hydroxyquinones in alkaline solution are useful aids to structural determination [35].

Formation of pink, red or violate color with aqueous sodium hydroxide or ammonia indicates the presence of quinones [45]. Anthraquinones can be distinguished from benzoquinones and naphthaquinones as they usually give red solutions on reduction in alkaline solution (zinc in aqueous sodium hydroxide) [46]. The orientation of hydroxyl groups of hydroxyanthraquinones can be predicted by observing the characteristics colors given when they are treated with methanolic magnesium acetate [47].

5.2. Spectroscopic Methods

I. UV (Ultraviolet Spectroscopy)

Anthraquinones consist almost entirely of polyhydroxy or alkoxy derivatives, and the influence of these substituents dominates the spectra [35]. When a carbonyl group is congugted with an ethylenic linkage both the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ bands undergo abthochromic shift to the region 220 – 260 nm and 310 – 330 nm respectively. The conjugation of a double bond with the π system of the carbonyl group lowers the energy differnce between the π and π^* orbitals. Anthraquinones shows intense benzenoid absorption at ca 250nm, medium absorption at ca 322 nm, a strong quinonoid electron transfer bands are seen at ca 270 – 290 nm accompanied by a weak quinonoid absorption at ca 405 nm. These areas of selective absorption are characteristic and the pattern in the ultraviolet region is not seriously affected by substitutions [35].

Hydroxyl groups in positions 2, 6 and 7 give rise to a stronger red shift bands than those with hydroxyls in 1,4 or 5 positions probably because in the former a hydroxyl group is para to one of the carbonyl groups [49]. The UV-Vis spectra of 1, 8-dihydroxy-anthraquinones show a peak at 430-450 nm and those of 1,4-dihydroxyanthraquinones exhibit absorption at 470-500 nm. This is also reflected in the color of anthraquinones, where 1,8-dihydroxyanthraquinones are yellow or orange and 1,4-dihydroxy-anthraquinones are red. Additional alpha hydroxylation results in a further red shift of the long wave length absorption [35].

II. IR spectroscopy

The carbonyl frequencies of quinones are useful diagnostic aids in structure determination and have been studied extensively. The carbonyl stretching vibrational absorption region frequencies are important in indicating the presence of the fundamental anthraquinone unit and the hydroxylation pattern. The stretching vibrations of the carbonyl groups of anthraquinnoes will show profound changes in the frequencies and intensity, if they have a hydroxyl groups in the alpha position. The carbonyl absorption of 9,10-anthraquinones with no alpha hydroxyl group falls at around 1678 cm^{-1}. The stretching vibration of anthraquinone, having positions 9 and 10 in keto form, shows a second carbonyl band at lower frequency if it contains hydroxyl group in the alpha position due to conjugation and chelation. From the study of several anthraquinones, Briggs and co-workers [35], found the correlation between the carbonyl frequency range and the number of alpha hydroxyl groups as shown in the Table 6.

Table 6. Carbonyl frequencies of hydroxyanthraquinones [46]

Number of α-OH groups	υCO (Nujol) cm^{-1}
None	1678-1653
1	1675-1647 and 1637-1621
2 (1,4- and 1,5-)	1645-1608
2 (1,8-)	1678-1661 and 1626-1616
3	1616-1592
4	1592-1572

In the hydroxyl region of the IR spectra, if a hydroxyl group at a beta position is present on the anthraquinone nucleus , a sharp hydroxyl stretching band will be apparent between 3600 and 3150 cm^{-1}. Alpha hydroxyanthraquinone shows no noticeable absorption in the hydroxyl regions but display a broad and weak absorption band center at ca2700cm-1 corresponding to the stretching frequency of a chealted hydroxyl band [46]. Chelated carbonyls can be recognized by their displaced carbonyl absorptions together with the downward shift in hydroxyl frequency [35].

III. NMR

Proton NMR and ^{13}C NMR spectra are powerful tools in the structural elucidation of anthraquinones. Analysis of chemical shifts and splitting patterns of anthraquinones give useful information for structural assignment. In proton NMR the α and β protons 9, 10-anthraquinone give multiplets centered at 8.07 and 7.67 ppm, respectively and are modified by substitution. The hydroxyl protons on 1,4,5, and 8 positions are easily distinguishable from other types of hydroxyl positions by their appearance at unusually low field resonance between 11 and 14 ppm or above.

Information about orientation of substituents around the aromatic ring system can be obtained from the chemical shift positions of aromatic protons. Since many quinones are phenolic, calculation of theoretical chemical shifts by the use of shielding parameters compiled for phenolic compounds can be used to predict the chemical shift of the aromatic protons and hence orientation of substituents[48]. Splitting patterns and coupling constants are also useful aids for the determination of orientation of aromatic substituents by PNMR [45]. Coupling constants show wide variations depending on bond angles and bond hybridization. However, aromatic coupling constants are almost constant. *Ortho* coupling constant (J_o) is usually around 7-9 Hz, *meta* coupling constant (J_m) is 2-3 Hz and *para* coupling constant (J_p) is about 1Hz. Any single aromatic proton may exhibit only one of seven possible first-order splitting patterns, as depicted in Table 7. Anthraquinones of the chrysophanol type show ortho-meta and di-ortho splitting patterns for the protons at position 5 or 7 and 6, respectively.

Table 7. Peak multiplicity nomenclature [45]

Coupling	Splitting pattern
ortho	broad doublet
di-*ortho*	broad triplet
meta	narrow doublet
di-*meta*	narrow triplet
ortho-meta	doublet of doublets
di*ortho-meta*	triplet of doublets
ortho-di*meta*	doublet of triplets

They also show the *meta* multiplicity for the protons at position 2 and 4, where the signals are broadened by the allylic coupling with the methyl protons at position 3. Emodin **(7)** or physcion **(6)** type anthraquinones show only the *meta* coupling patterns. The other splitting patterns mentioned in Table 7 are not common in anthraquinones.

In ^{13}C NMR spectra of anthraquinones, carbon 9 and 10 are easily recognized by their resonance occurring between 180 and 195ppm while carbons bearing hydroxyl groups appear at about 160ppm [49].

VI. Mass Spectrum

The molecular ion almost invariably forms the base peak. Anthraquinone itself undergoes successive elimination of two molecules of carbon monoxides to give strong peak at m/e 180(M-CO) and 152 (M-2CO) and strongly doubly charged ions at m/e 90 and 76 which corresponds to the molecular ions of fluorenone and biphenylene, respectively. The spectra of derivatives follow the same pattern with additional peaks appropriate to the substituents and their α- or β- orientation. 2-Hydroxyanthraquinones shows more intense M-CO and M-OH peaks than does the chelated 1-iosmer and both spectra have a peak at m/e 140 corresponding to the loss of three molecules of CO, the third arising from phenolic group in the normal manner. Dihydroxy anthraquonones behave similarly and a peak at M-4CO (m/e 128) may be the molecular ion of napthalene[35].

6. Objectives

The main objective of this project is isolation and structural elucidation of secondary metabolites, from the pods of *Senna occidentalis* from Ethiopia.

7. Senna occidentalis

- Common Name (Vernacular names) : Coffee Senna (English), Senamachi (collection cite), Asene Meka (Oromiya, Kemisea)[50], Sachara (Oromiya), Shuna-Shuna (Somali) [9],
- Scientific Name: *Cassia occidentalis* L (1753), *Senna occidentalis* (L.) Link (1831): Initially classified in cassia genus by Carl Linneous (1753) and then the classification was revised by Link in 1831 to be in the genus Senna [9].
- Family: Leguminosae (Fabaceae), Bean Family
- Sub Family: Caesalpinoideae

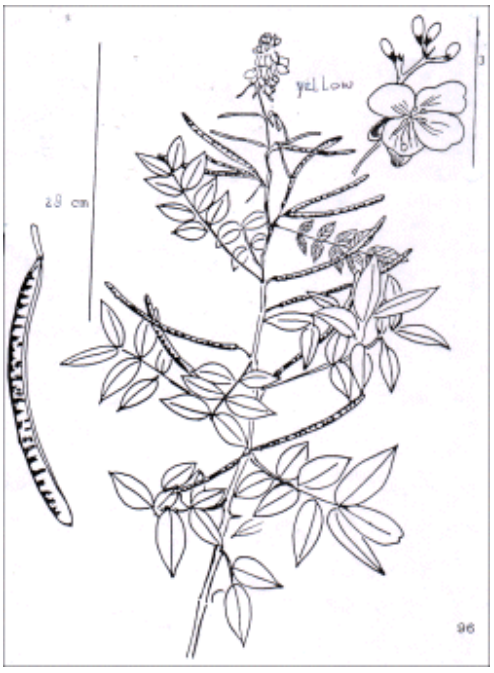


Figure 6. Parts the plant *Senna occidentalis* [53]

Coffee senna is an annual or short-lived perennial shrub which grows up from 0.2 to 2 m high. The stems are smooth, often woody at the base and have lengthwise grooves. The leaves are compound, consisting of three to five pairs of leaflets along a 15 to 20 cm rachis. The leaflets are smooth, have a pointed tip and rounded base, and are 3 to 5 cm

long and 2 to 3 cm broad. The compound leaves are alternate on the plant stem, and have a large, dark gland where they join the stem. Coffee senna has yellow, five petaled, pea-type flowers about 0.9 – 1.5 cm long. The flowers grow in clusters on short stalks near the end of branches. Seeds are produced in smooth, flattened beanlike pods about 8 – 13 cm long and 0.5 - 1 cm wide. The pods grow upwards from the stem and curl towards the branch tip(Slightly up curved). The somewhat flattened black seeds are about 4.5 - 5 mm x 3.75 – 4.5mm and are shiny and smooth[9].

Coffee senna is a native of tropical America. Usually weed of cultivation, road side and waste place [8,9], also in wooded grassland and near lakes or streams 500 - 2400 m. The plant is mainly distributed in low lands of Afar, Tigray, Gonder, Welega, Arsi, Harari, and Southern part[9].

Senna occidentalis is regarded as famine food [9] by the African community and is typically only consumed when nothing else is available. Preliminary assessment of nutritional value showed that its leaves do have high nutritional value and it is recommended to introduce it into cultivation [51].

8. Experimental Part

8.1 General

Instruments:

^{1}HNMR and ^{13}CNMR: Bruker Avance at 400 MHz

UV: Genesy's 2PC UV-Vis Scanning spectrometer (200 – 1000cm^{-1})

IR: Perkin-Elmer BX Infrared Spectrometer (4000 – 400 cm^{-1})

Melting Point: Mettler Toledo melting point apparatus

Chromatography:

Column Chromatography: Silica gel 60, particle size 0.063 – 0.200 mm (70 – 230 mesh ASTM)

Analytical TLC: silica gel 60 PF_{254} (fluka) coated on aluminum sheet, 0.20 thickness.

Preparative TLC: silica gel 60 $PF_{254+366}$ (merk) 1 mm, 0.75 mm, 0.50 mm (70 – 230 mesh ASTM)

Sephadex LH -20 (Pre packed Column)

Chromatotron: Chromatotron Model 7942T, Harrison Research.

Impergnation of silca gel (both column and TLC): with 5% oxalic acid

Spray reagent: 5% KOH in ethanolic solution, 10% (w/v) Ethanolic Solution of KOH (Borntrager Reagent [3]), Vanilline 5% in sulphuric acid

Solvent Systems:

Solvent 1: Petroleum ether

Solvent 2: Chloroform

Solvent 3: Methanol

Solvent 4: Chloroform: Methanol (9:1)

Solvent 5: Chloroform: Ethyl Acetate (4:1)

Solvent 6: Chloroform: Methanol (2:1) for sephadex

Solvent 7: Petroleum ether: Ethyl Acetate (9:1)

Solvent 8: Petroleum ether: Ethyl Acetate (9.5:0.5)

Solvent 9: Petroleum ether: Ethyl Acetate (4:1)

Solvent 10: Chloroform: Methanol (9.5:0.5)

Solvent 11: Petroleum ether: Ethyl Acetate (1:1)

Plant material:

The pods of *Senna occidentalis* were collected on the road side between Asebot and Mesio on March 07, 1992. The specimen of the plant is deposited at the national Herbarium of AAU with a voucher number- 213.

8.2 Extraction and Isolation: Chloroform Extract

The dried pod was ground and sieved using the local instruments available in the chemistry garden AAU, Science Faculty. The mass of the plant material was 700 g. Initially we need to optimize the steps to be used in the extraction and isolation. 100 g of the dried and ground plant material was soaked in 5% acetic acid (by volume) 250 ml, and allowed to dry by air. Then the dried sample was successively extracted with Solvent 1 (4 x 0.5L) after soaking for 24hrs, Solvent 2 (6x0.5L) after soaking for 24 hrs and then with solvent 3 (5x0.5L) after soaking for 24hrs.

The chloroform (Solvent 2) extract was concentrated under a reduced pressure to yield 0.8 g (0.8%). Then the whole part was subjected to column chromatography which was packed with 30 g of silica gel impregnated with 5% oxalic acid after being adsorbed in 3 g of silica jel, and eluted with solvent 3. 24 fractions each ca15 ml and 2 fractions each 100 ml were collected. These fractions are grouped into 11 based on TLC (solvent 5) similarities of the fractions collected. These groups are again concentrated and checked for characteristic spots on TLC (solvent 5). The 11 (A-K) groups are recombined according to TLC(solvent 5) similarities in to 5 and labeled as CEO-1– 5(CEO stands for chloroform extract for optimization). After looking for the characteristic spots (solvent 5) and their respective masses CEO–1 (271 mg), CEO–3 (39 mg) , and CEO– 4 (40 mg) were successively subjected to Sephadex (Solvent 6, pre-packed).

CEO-1 (271 mg) was subjected to column chromatography on sephadex LH-20 (solvent 6) and 8 fractions collected. Fraction 3(62mg) and fraction 6(64 mg) showed each one characteristic spot (solvent 9). The other fraction showed more than one characteristic

spot and the masses are less then 15mg in each case. Both fraction 3 and 6 are separately applied on preparative TLC on three plates each. CEO-131 and CEO-161 are isolated respectively. Both are sent for NMR analysis. The spectra of both were not that much clear to be interpreted. CEO-161 will be discussed in the major step repeating itself. The NMR spectra of CEO-131 were done again and seem to be a bianthraquinone.

CEO – 3 (37 mg) was also subjected to column chromatography on sephadex LH-20 (Solvent 6) and five fractions collected and checked with TLC (Solvent 11) for better separation of characteristic compounds . Fraction 3 was washed several times with n-hexane and sent for NMR analysis as CEO-330. Fractions 4 and 5 are combined and applied on a single plate of Preparative TLC (Solvent 11), a sample with a mass of less than 1 mg was obtained. CEO-4 (40 mg) was also subjected to sephadex and another five fractions collected. The last fraction showed characteristic spot indicating the presence of trace anthraquinones but too small to be purified further.

After looking through the optimization step the remaining 600g of the plant material (pod) was soaked in 5% acetic acid (by volume) 2L and allowed to dry for a week. Then the dried and acidified plant material was extracted successively with petroleum ether (5x3L), chloroform (6x2.5L) and methanol (4x2L) each time after soaking fro 14 hours in the respective solvent. See the extraction summary in the result and discussion part.
The concentrated and dried chloroform extract was found to be 1.5 g and looks a brown gummy solid. The whole amount of the extract was applied on Column with silica gel impregnated with oxalic acid (5%). The silica gel used was 60 g and 5 g of silica gel were also used for adsorbing the dried sample. After application elution took-over with chloroform and 14 fractions each 25 ml were collected. Based on TLC(solvent 9), fractions 3, 8 and 13 are found to be important fractions both on their amount and characteristic spots after being sprayed with 5%KOH ethanolic solution.

Fraction 3 (315 mg) with one characteristic spot and other minor spots was labeled as CE-SOP-3 (CE-SOP stands for chloroform extract of *Senna occidentalis* Pod) and subjected to sepahedx (pre-packed), eluted with solvent 6. Seven fractions were

collected each 15 ml. Fractions 2,3,4 found to be similar with each other and also have only single characteristic anthraquinone spot (solvent 9) after being sprayed with 5%ethanolic KOH. The combined fraction was dried and dissolved in chloroform which is then filtered to remove non-dissolving substances (most probably oxalic acid used for impregnation of the silica gel). The filtered fraction labeled as CE-SOP-32 was then freed of the solvent and weighed to be 80 mg.

While preparing CE-SOP-32 for NMR the whole portion could not be dissolved in $CDCl_3$, but upon heating it dissolves and a solid precipitate forms after awhile in the NMR tube. We decide to check the spot with some other solvent system. When TLC checked with a different solvent system (solvent 7 and 8), the later one shows a better separation and one anthraquinone spot together with others with grater Rf values observed after spraying with vaniline(5% in sulphuric acid) and heated for some minutes. Using this solvent system CE-SOP-32 was applied on smaller column with 10g silica gel. After applying the dissolved sample in solvent 8, eluted with the same solvent system and 14 fractions collected. These fractions were combined in to 3 groups based on TLC check with the same solvent system. The second group of fractions with a single characteristic spot was combined and freed of solvent. The dried sample was found to be yellow solid weighing 4mg. By dissolving in $CDCl_3$ the sample was sent for NMR analysis as CE-SOP-322 and the spectrum was interpreted for the anthraquinone physcion. CEO-161 from the optimization step and CE-SOP-322 were found to be similar up on TLC (solvent 8) comparison.

Fraction 8 (CE-SOP-8) was also subjected to sepahdex (pre-packed) and eluted as before. Five fractions collected. Fractions 2, 3, 4 showed one major spot and two minor spots in common (solvent 2) and combined and concentrated and labeled as CE-SOP-82. This sample was applied on Chromatotron and eluted with solvent 2 to separate the major spot from the mixture and could not be separated.

Fraction 13 (CE-SOP-13) was also applied on sephadex (pre-packed) and eluted as before. Eleven fractions collected and two groups comprising fractions 4,5 and fractions

8,9,10 are found to be significant with two characteristic spots. Fractions 4,5 (57 mg) were labeled as CE-SOP-13 -4 and fractions 8,9,10 (99 mg) labeled as CE-SOP-8. Selecting solvent system for CE-SOP-13-4 was found to be difficult. After selecting a better solvent CE-SOP-8 was applied on a small column with 10g of silica gel and eluted with solvent 10. Eight fractions were collected and grouped in to four after checking TLC with the same solvent. The second group was freed of solvent and gave an orange solid (5 mg). The third group have other spots and a common spot with the second group and left alone. The second group was freed of solvent and sent for NMR analysis as CE-SOP-13-80. It was not readily soluble in $CDCl_3$. the spectrum was not found to be good. After looking for the ^1H NMR of CE-SOP-13-80, we decided to wash it with n-hexane. The sample was washed 3 times with n-hexane. Light yellow dirt removed and then the remaining sample was freed of solvent and sent for NMR analysis as CE-SOP-13-80A. A better spectrum obtained and interpreted to be emodin (an orange yellow solid). This ample was observed to dissolve in acetone readily.

(a) (b)

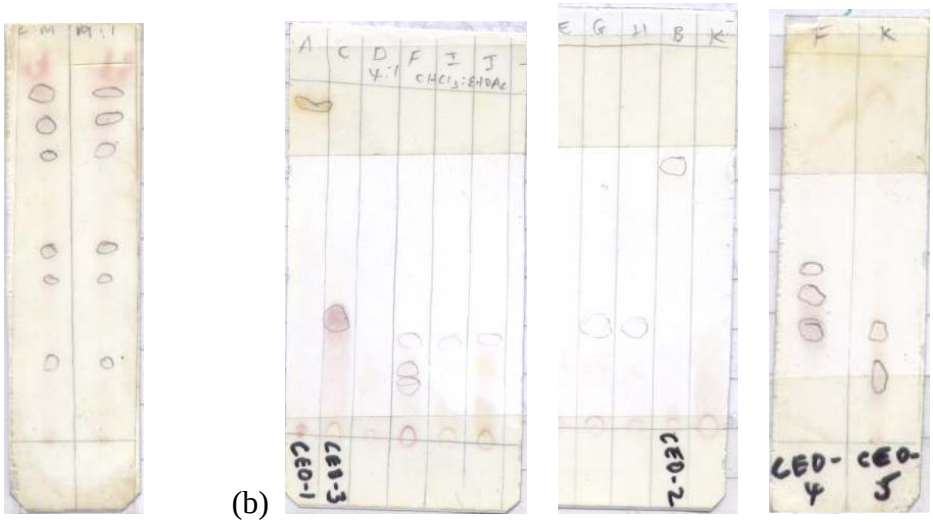


Figure 7. TLC Spots. (a) Chloroform extract(in $CHCL_3$: MeOH (19:1)), (b) first fractions of CC(Solvent 5)

8.3. Methanol Extract

The methanol extract of the pod of *Senna occidetnalis* was checked for significant characteristic spot with different solvent systems and no significant characteristic spot was found.

9. Result and Discussions

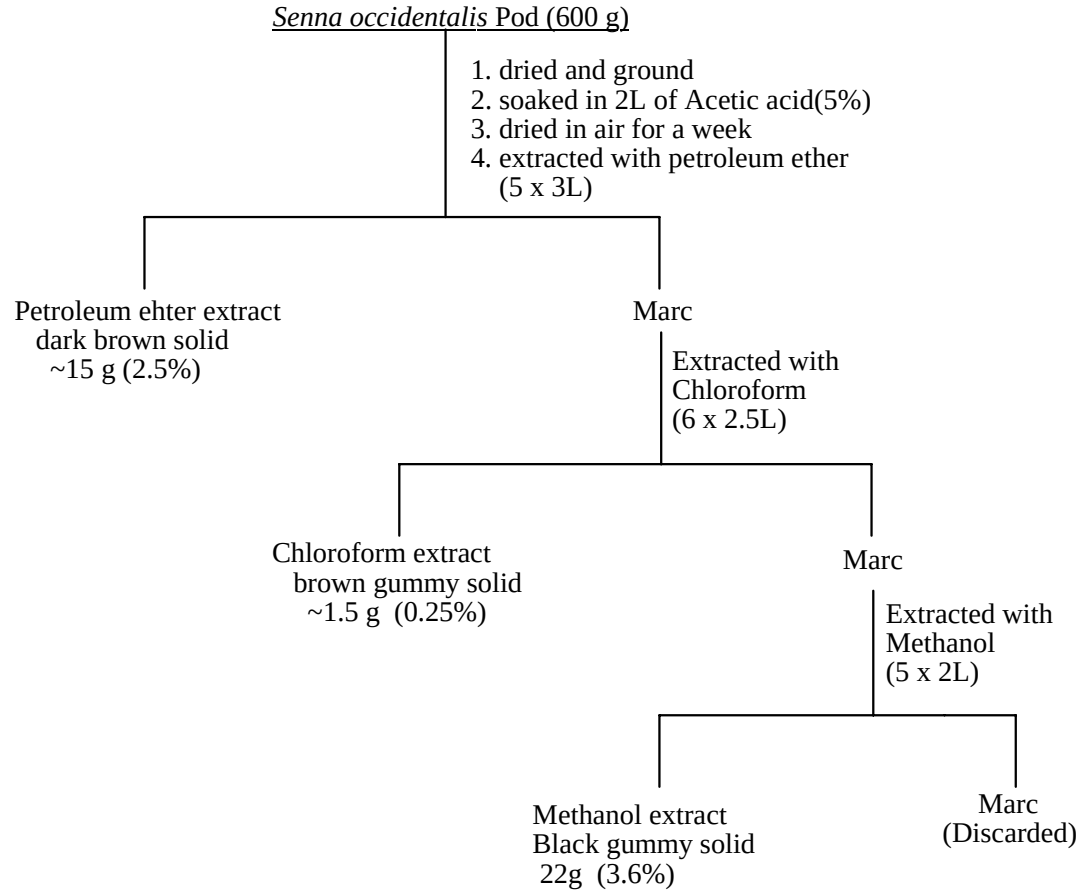
9.1 General

No investigation is reported on the pod of *Senna occidentalis* except the one by Singh M. and Singh J. for the isolation of two flavonoid glycosides from its ethanolic extract [55]. The chloroform extract of the pod of *Senna occidentalis* was the major area of attention. Initially 100g of the dried and powdered plant material (pod) were used for optimizing the procedures obtained from previous works [10,49, 52].

Soaking of the ground pod with 5% acetic acid was done to increase the ease of separation of polar components during extraction. Similarly impregnation of the silica gel with 5% oxalic acid was intended to increase the ease of chromatographic separation by increasing the acidity of the silica gel and helping in the easy elution of the acidic anthraquinones.

The pod of *Senna occidentalis* was extracted successively with petroleum ether (defatting), chloroform and methanol. The chloroform extract which looks brown and gummy was found to be 0.25 %. It is smaller when compared with that of the optimization step. Extraction in the optimization step and the main step are almost the same except for the transition between the deffating and the chloform extraction. This transition was early in the case of the optimization step as it is reflected on the % decrease of chloroform extract in the main step.

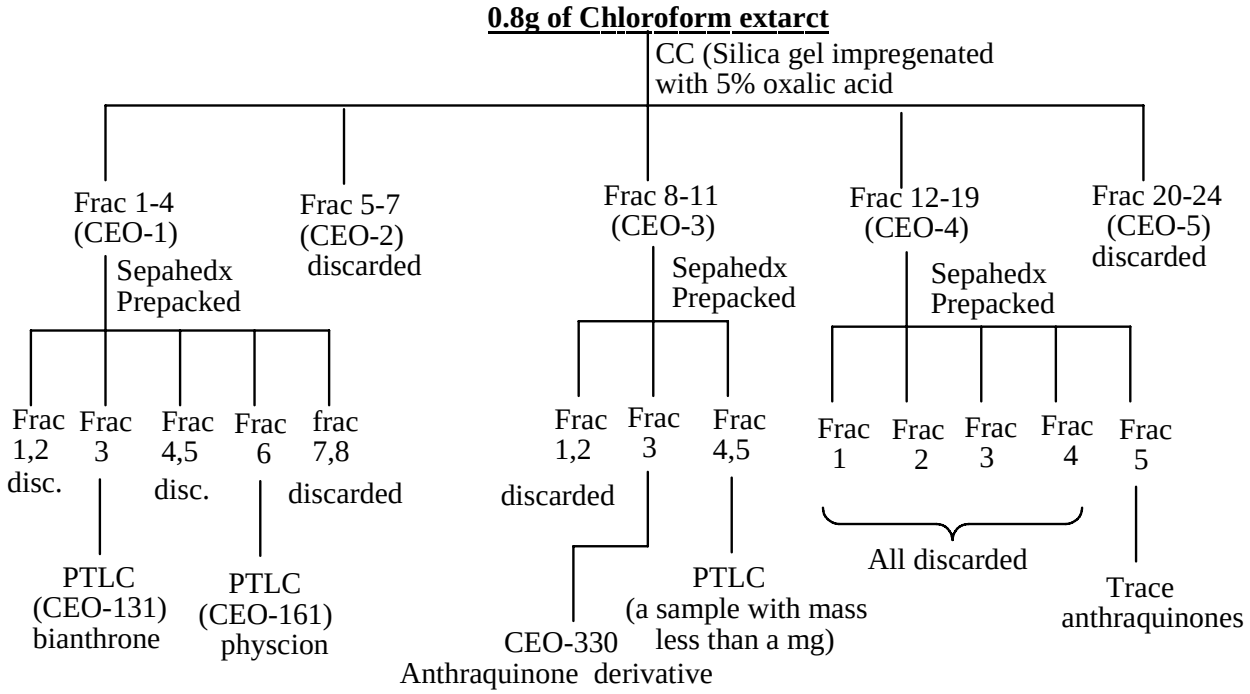
After acid hydrolysis and defatting steps, the mass and spots on TLC (thin layer chromatography) for both chloroform and methanol extracts were compared. Different solvent systems were also tested for better spots on TLC. This helps us to choose the best solvent system for column application of chloroform extract. The extraction steps are summarized as in Scheme 4.



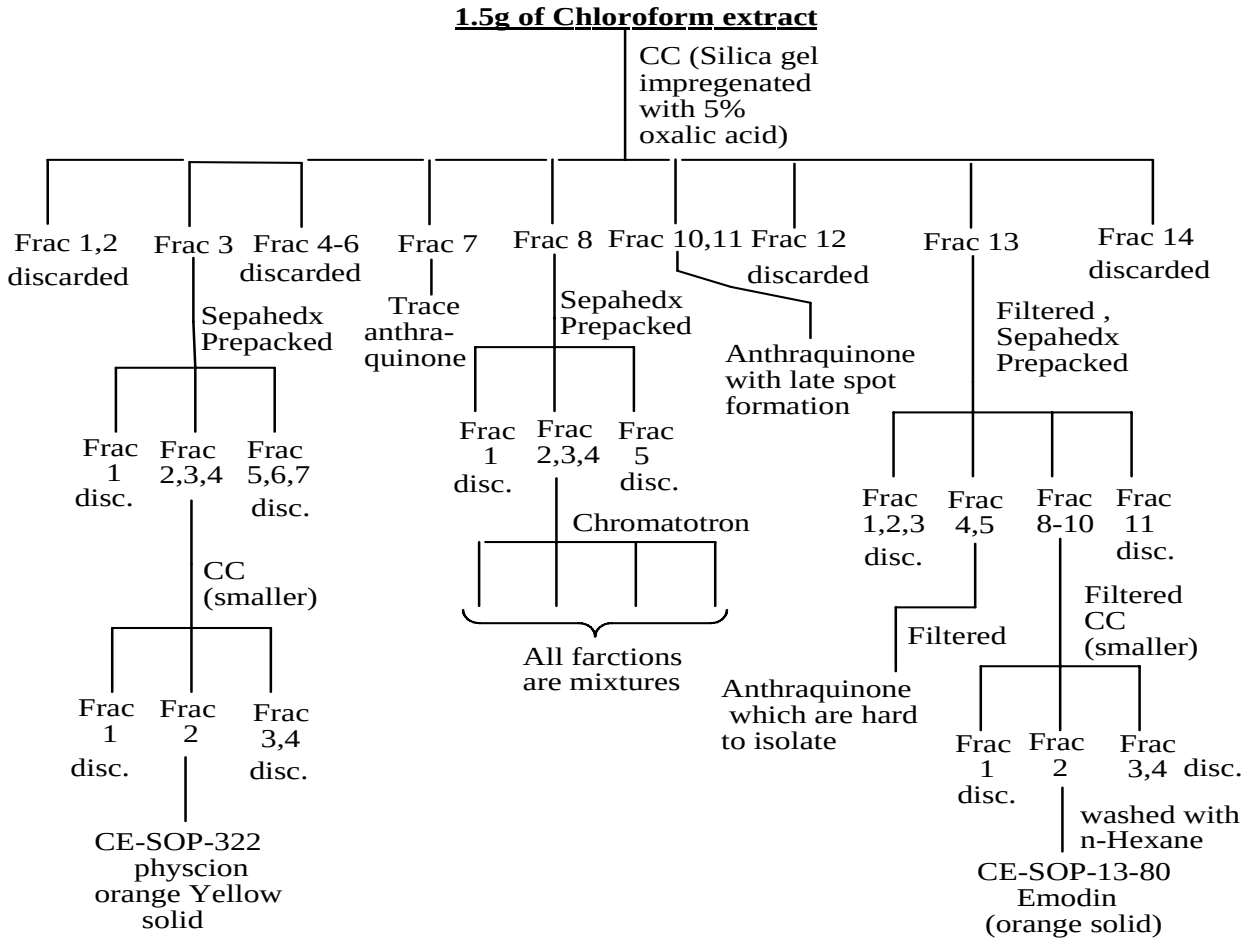
Scheme 4. Extraction Summary

9.2 Isolation and Purification of Anthraquinones from the Chloroform Extract

The brown gum chloroform extract was subjected to column chromatography on silica gel which is impregnated with 5% oxalic acid. Then follows gel filtration using sephadex (which was pre-packed) was done. Finally for purification preparative TLC, Chromatotron and smaller column were used. The isolation and purification steps are summarized as in Scheme 5 and 6.



Scheme 5. Isolation and purification in the optimization step



Scheme 6. Isolation and purification in the main step.

Fraction 7 and fraction 10, 11 from the first column shows characteristic spots while spraying with 5% KOH ethanolic solution but their amount was too small to proceed further. Especially fraction 10, 11 are forming the characteristic spots delayed indicating the presence of anthraquinones which are substituted on one of the aromatic ring only.

From this work two anthraquinones and a bianthrone and anthraquinone derivative which are partially characterized were generated. The major tackles throughout this work are:

- ☞ Repeated filtration to remove solid substances most probably the oxalic acid used for impregnating the silica gel for chromatographic separation.
- ☞ Solvent tricks with the presence of fat substances with in the extract. Most likely polar fatty substances with nearly similar R_f values were overlapping on the TLC spots of the characteristic spots.
- ☞ The failure of the chromatothron to separate the mixture applied which may be attributed from the higher R_f value of the component to be separated and the presence of some oxalic acid residue. The R_f is some times higher in the chromatotron than on the regular TLC plates as discussed on the operation manual as troubleshoot. The mass of the sample may also be exaggerated due to the presence of oxalic acid residue and even the presence of this residue may affect the separation.

We have tried to over come with some of these problems and minimized their effects in our work.

The sample applied on the chromatotron was similar on TLC with CEO-131 (see Figure 8) from the optimization step whose partial structure will be presented latter.

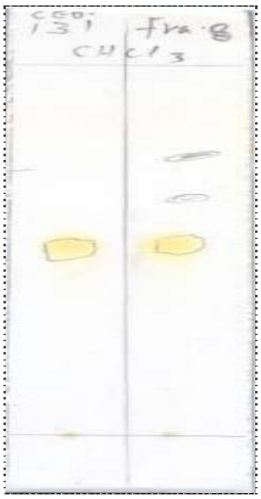
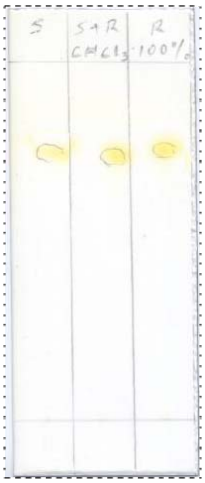


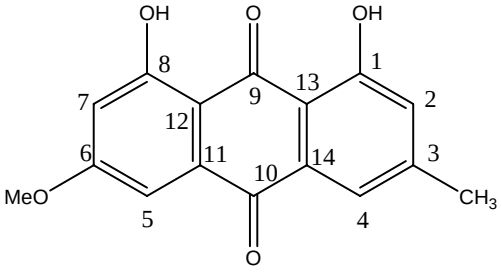
Figure 8. TLC spots for comparison of fraction 8 and CEO-131

9.3. Characterization of Anthraquinones from the Chloroform Extract

Physcion (6)

Compound **(6)** was an orange yellow solid that turned to red on a TLC plate upon spraying with 5% KOH in ethanolic solution, which is a characteristic color change for hydroxyl anthraquinones. It also displayed UV absorption at λ_{max} 264, 288 and 439 nm, characteristic of anthraquinones [35]. The IR showed bands at 1736 and 1629 cm^{-1} corresponding to the unchelated and chelated carbonyl carbons, respectively. The ^{1}H NMR showed two chelated hydroxyl resonances at δ 12.36 and δ 12.16, two broad singlet at δ 7.11 and δ 7.66, assigned to the protons at C-2 and C- 4 respectively, two protons at δ 6.72 and δ 7.40 representing the protons at C-7 and C- 5 respectively, a methoxy protons at δ 3.96, and a methyl group attached to aromatic ring resonates at δ 2.48. The ^{1}H NMR data is consistent with that reported for physcion in the literature [10] (Table 8). TLC comparison with reference in $CHCl_3$ (see below) also confirms for physion.





Physcion

Table 8. Comparison of the observed ^{1}H NMR spectral data with reported value of physcion.

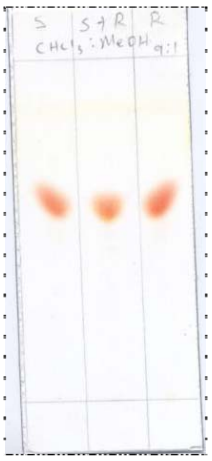
Proton	Observed data (400MHz , $CDCl_3$) δ in ppm	Reported data [10] (400MHz , $CDCl_3$) δ in ppm
1-OH	12.16 *s*	12.1 *s*
2-H	7.11 *brs*	7.05 *brs*
3-Me	2.48 *s*	2.40 *s*
4-H	7.66 *brs*	7.6 *brs*
5-H	7.40 *d*, *J*=2.5 *Hz*	7.35 *d*, *J*=3 *Hz*
6-OMe	3.96 *s*	3.90 *s*
7-H	6.72 *d*, *J*=2.5 *Hz*	6.5 *d*, *J*=3 *Hz*
8-OH	12.36 *s*	12.3 *s*

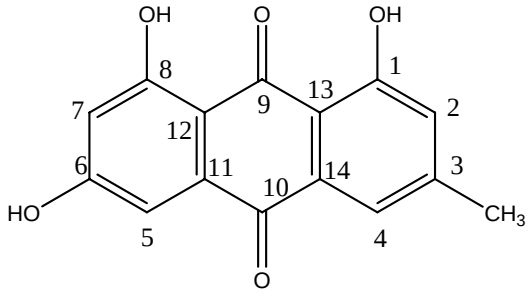
Physion and emodin occur together and it has been established that physion is derived from emodin in the cultures of *Aspergillus parasiticus* with the aid of labeled emodin[36].

Emodin (7)

Compound **(7)** was obtained as an orange solid that turned to purple red on a TLC plate upon spraying with 5% KOH in ethanolic solution, which is a characteristic color change for hydroxyl anthraquinones. It displayed UV absorption at λ_{max} 263, 285 and 439 nm, characteristic of anthraquinones [35]. The IR showed bands at 1625 cm^{-1} corresponding to the carbonyl carbons. ^{1}H NMR was done using $CDCl_3$ and Acetone d_6. Both gave comparable spectra. The ^{1}H NMR (Acetone d_6) shows two chelated hydroxyl

protons resonating at δ 12.07 and δ 12.20, two meta coupled doublets at δ 6.66 and δ 7.26, two meta coupled broad singlet signals at δ 7.15 and 7.57, and one methyl group at δ 2.48 are characteristic of emodin. TLC comparison with reference in $CHCl_3$: MeOH (9:1) (see below) also confirms for emodin.





Emodin

Table 9. Comparison of the observed ^1H NMR spectral data with reported value of emodin.

Proton	Observed data (400 MHz , $CDCl_3$) δ in ppm	Observed data (400 MHz, Acetone d_6) δ in ppm	Reported data [45] (400 MHz, Acetone d_6) δ in ppm
1-OH	12.13 *s*	12.07 *s*	12.09 *s*
2-H	7.12 *brs*	7.15 *brs*	7.16 *brs*
3-Me	2.47 *s*	2.48 *s*	2.48 *s*
4-H	7.66 *brs*	7.57 *brs*	7.59 *brs*
5-H	7.31 *d*, *J*=2.38 *Hz*	7.26 *d*, *J*=2.42 *Hz*	7.27 *d*, *J*=2.4 *Hz*
6-OH	----	----	----
7-H	6.69 *d*, *J*=2.38 *Hz*	6.66 *d*, *J*=2.42 *Hz*	6.68 *d*, *J*=2.4 *Hz*
8-OH	12.31 *s*	12.20 *s*	12.21 *s*

The ^{13}C NMR spectrum (acetone d_6) data of emodin (see Table 10), displayed 15 carbon resonance and the DEPT spectral data showed the absence of methylene groups, the presence of one methyl group at δ 21.2, four aromatic C-H protons at (δ 124.1, 120.6, 108.8, and 107.9), and ten quaternary carbons is consistent with emodin structure.

Table 10. Comparisons of ^{13}C NMR (400MHz) and DEPT 135 spectral data of emodin in acetone d_6.

Position of carbon	^{13}C NMR data, Acetone d_6 (δ in ppm)	DEPT 135 data , Acetone d_6 (δ in ppm)	^{13}C NMR [Lit [45]] data, Acetone d_6 (δ in ppm)	Remark
CH_3	21.2	21.2	21.1	CH_3
C-7	107.9	107.9	107.9	C-H
C-5	108.8	108.8	108.8	C-H
C-12	109.6	---	109.6	Quaternary
C-13	113.6	---	113.6	Quaternary
C-4	120.6	120.6	120.6	C-H
C-2	124.1	124.1	124.0	C-H
C-14	133.4	---	133.3	Quaternary
C-11	135.4	---	135.7	Quaternary
C-3	148.7	---	148.6	Quaternary
C-1	162.4	---	162.4	Quaternary
C-8	165.4	---	165.4	Quaternary
C-6	165.5	---	165.4	Quaternary
C-10	181.3	---	181.2	Carbonyl
C-9	190.9	---	190.8	Carbonyl

These data along with UV, IR and melting point data, which are in accord with literature value [35], explain the structure of emodin. Emodin is the precursoure of numerous mould metabolites including the ergochromes, geodin and dihydrogeodin formation of which involves enzymatic oxidative cleavage of ring B [36]. As it was first isolated in 1858, several synthetic procedures are now available that yield emodin in moderate to good yield [36].

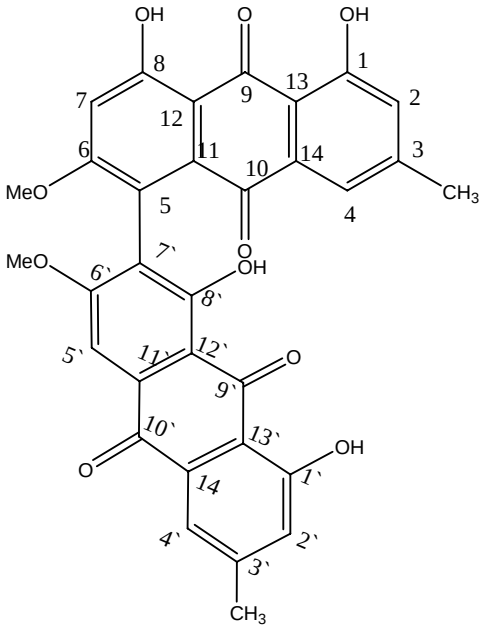
CEO- 131 (5,7`-biphyscion)(37)

Compound **(37)** was obtained as an orange yellow solid that turned red on TLC plate upon spraying with 5%KOH in ehtanolic solution. This color change is characteristic for hydroxyl anthraquinones[35]. It displayed UV absorption at λ_{max}: 263, 284 and 440 nm, characteristic of anthraquinones [24]. The IR showed bands at 1725 and 1629 cm^{-1} corresponding to the un-chelated and chelated carbonyl carbons, respectively. The ^{1}H NMR spectrum of compound **(37)** indicated the presence of four chelated hydroxyl groups (δ 13.07, 12.30, 12.14, 12.10) one of which is strongly chelated, six aromatic C-H protons (δ 7.72, 7.56, 7.40, 7.13, 7.11, 6.84), two methoxy groups (δ 3.90, 3.85) and two aromatic methyl groups (δ 2.50, 2.33) (see Table 11).

Table 11. Comparison of the observed ^{1}H NMR spectral data with reported value of 5,7`-biphyscion.

Proton	Observed data (400 MHz , $CDCl_3$) δ in ppm	Reported data [54] (400 MHz , $CDCl_3$) δ in ppm
1-OH	12.14 *s*	12.10 *s*
2-H	7.11 *s*	7.04 *br d J*=2.0
3-Me	2.33 *s*	2.35*s*
4-H	7.40 *s*	7.42 *br d J*=2.0
5-H	-----	------
6-OMe	3.85 *s*	3.82 *s*
7-H	6.84 *s*	6.82 *s*
8-OH	12.10 *s*	12.05 *s*
1`-OH	12.30 *s*	12.20 *s*
2`-H	7.13 *s*	7.06 *br d J*=2.0
3`-Me	2.50 *s*	2.45 *s*
4`-H	7.72 *s* + impurity	7.67 *br d J*=2.0
5`-H	7.56 *s* + impurity	7.57 *s*
6`-OMe	3.90 *s*	3.85 *s*
8`-OH	13.08 *s*	13.10 *s*

As the protons at δ 7.72, 7.56 are masked with impurity it is not possible to predict the structure fully. Most of the ^{1}HNMR chemical shifts agree with the literature value reported [54]. With these data and UV, IR and melting point data it is possible to suggest tentatively the following structure. As the amount is too small it was not possible to further purify.



5,7`-biphyscion

CEO- 330 Anthraquinone derivative (38)

Compound **(38)** is reddish pigment which turned into deep red color upon spraying with 5% KOH in ethanol. Its ^{1}HNMR spectrum shows a three proton singlet at δ value of 2.20 due to aromatic methyl group, two three proton singlet at δ values of 3.95 and 3.73 due to methoxyl groups on aromatic ring and three one proton singlets at δ values of 12.41, 12.06, 10.24 due to aromatic hydroxyls which are expected to be chelated. In addition to this it shows one proton singlets in the aromatic region.

The IR spectrum shows an absorption band at 3436cm^{-1} due to aromatic hydroxyls and only one band in the region between 1600 and 1700cm^{-1} , at 1624cm^{-1} indicating both carbonyls are chelated .The maximum in the UV spectrum recorded in $CHCl_3$ is at 274 and 416nm. From these data one can suggest that the compound is a tri-hydroxy-dimethoxy-methylanthraquinione. On the basis of these spectral data no conclusion could be drawn about the substitution pattern of this compound.

10. Conclusion

Two anthraquinones were isolated and characterized from the pod of *Senna occidentalis* as physcion and emodin. These were not the only anthraquinones identified. There are a number of trace anthraquinones which show characteristic spots upon spray with 5% ethanolic KOH solution.

A dimeric anthraquinone was identified and isolated and identified tentatively as 5, 7'-biphyscion. Though it is not possible to propose a structure a trihydroxy-dimethoxy-methylanthraquinone was also isolated.

11. Spectral Data

Physcion (6): Orange yellow solid, m.p. 204-208°C (lit. [35] 207°C); R_f 0.70 in pet. ether: EtOAc (4:1); UV-Vis λ_{max} ($CHCl_3$) nm: 264, 288, and 439. IR υ_{max} (KBr) cm^{-1}: 3436, 2959, 2925, 1736, 1629, 1570, 1480, 1368, 1324, 1274, 1226, 1163, 1036, 850,757. ^{1}H-NMR ($CDCl_3$) δ: 2.48 (3H, *s*, CH_3), 3.96 (3H, *s*, -OCH_3), 6.72 (1H, *d, J*=2.5 *Hz,* H-7), 7.11 (1H, *brs*, H-2), 7.40 (1H, *d*, H-5, *J*=2.5 *Hz*), 7.66 (1H, *brs*, H-4), 12.16 (1H, *s*, 1-OH), and 12.36 (1H, *s*, 8-OH).

Emodin (7): Orange solid, m.p. 253-256°C (lit. [35] m.p. 255°C); R_f 0.30 in pet. ether: EtOAc (4:1); UV-Vis λ_{max} (CHCl3) nm: 263, 285, and 439. IR υ^{max} (KBr) cm^{-1}: 3476, 1625, 1479, 1384, 1342, 1272, 1227, 1174, 1098, 769. ^{1}H-NMR (acetone-d6,) δ: 2.48 (3H, *s*, CH_3), 6.65 (1H, *d, J* = 2.42 *Hz*, H-7), 7.15 (1H, *brs*, H-2), 7.26 (1H, *d, J* = 2.42 *Hz*, H-5), 7.57 (1H, *brs*, H-4), 12.07 (1H, *s*, -OH), and 12.20 (1H, *s*, -OH). ^{13}C-NMR (acetone-d_6) δ: 21.16 (-CH_3), 107. 99(C-7), 108.76 (C-5), 109.60 (C-12), 113.59 (s, C-13), 120.61(C-4), 124.08 (C-2), 133.37 (C-14), 135.37 (C-11), 148.70 (C-3), 162.41 (C-1), 165.39 (C-8), 165.47 (C-6), 181.30 (C-10), and 190.88 (C-9).

5,7`-biphyscion (37): Orange Yellow solid, m.p.255-260 °C (lit. [36] m.p. 255°C); R_f 0.40 in pet. ether: EtOAc (4:1); UV-Vis λ_{max} ($CHCl_3$) nm: 263, 284 and 440. IR υ_{max} (KBr) cm^{-1}: 3435 2924, 2852, 1725, 1629, 1383, and 623. ^{1}H-NMR ($CDCl_3$) δ: 2.33 (3H, s, 3-Me), 2.50 (3H, *s*, 3′ -Me), 3.85 (3H, *s*, 6-OMe), 3.90 (3H, *s*, 6′-OMe), 6.84 (1H, *s*, H-7), 7.11 (1H, *brs*, H-2), 7.13 (1H, *brs*, H-2′), 7.40 (1H, *brs*, H-4), 7.56 (1H, *s*, H-5′)+ impurity, 7.72 (1H, *brs*, H-4′)+ impurity, 12.14 (1H, *s*, 1-OH), 12.10 (1H, *s*, 8-OH), 12.30 (1H, *s*,1′ -OH), and 13.08 (1H, *s*, 8′-OH).

Compound (38): Red pigment R_f 0.30 in pet. ether: EtOAc (1:1); UV-Vis λ_{max} ($CHCl_3$) nm: 274 and 416. IR υ_{max} (KBr) cm^{-1}: 3436 2916, 2849, 1624, 1471, 1384, 1271, 1133, 718, and 621. ^{1}H-NMR ($CDCl_3$) δ: 2.20 (3H, *s*, Ar-Me), 3.95 (3H, *s*, Ar-OMe), 3.73 (3H, *s*, Ar-OMe), , 6.10 (1H, *s*, Ar-H), 6.56 (1H, *s*, Ar-H) 7.16 (1H, *s,* Ar-H), 7.61 (1H, *s,* Ar-H), 12.41 (1H, *s,* Ar-OH), 12.06 (1H, *s*, Ar-OH), and 10.24 (1H, *s,* Ar-OH).

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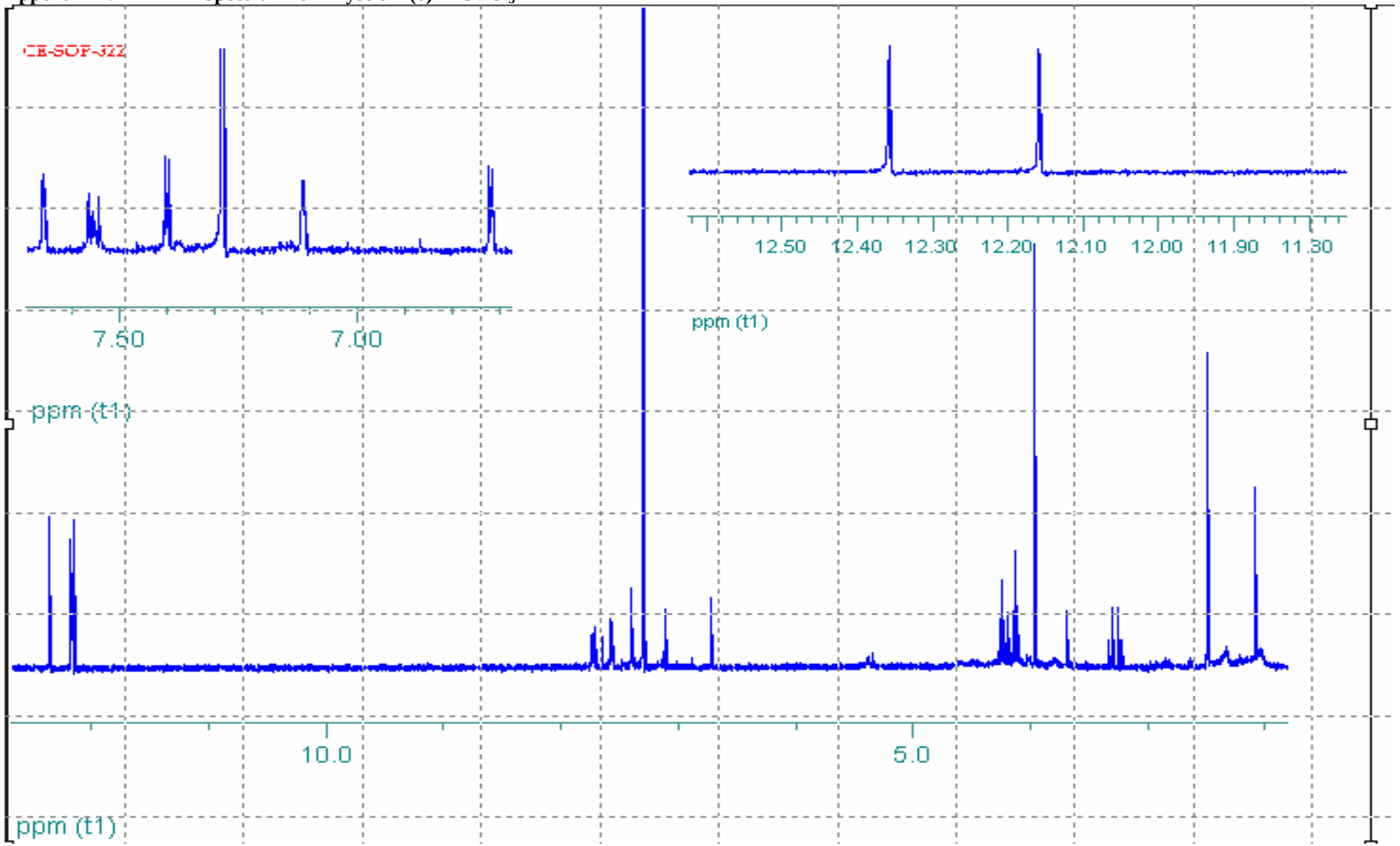
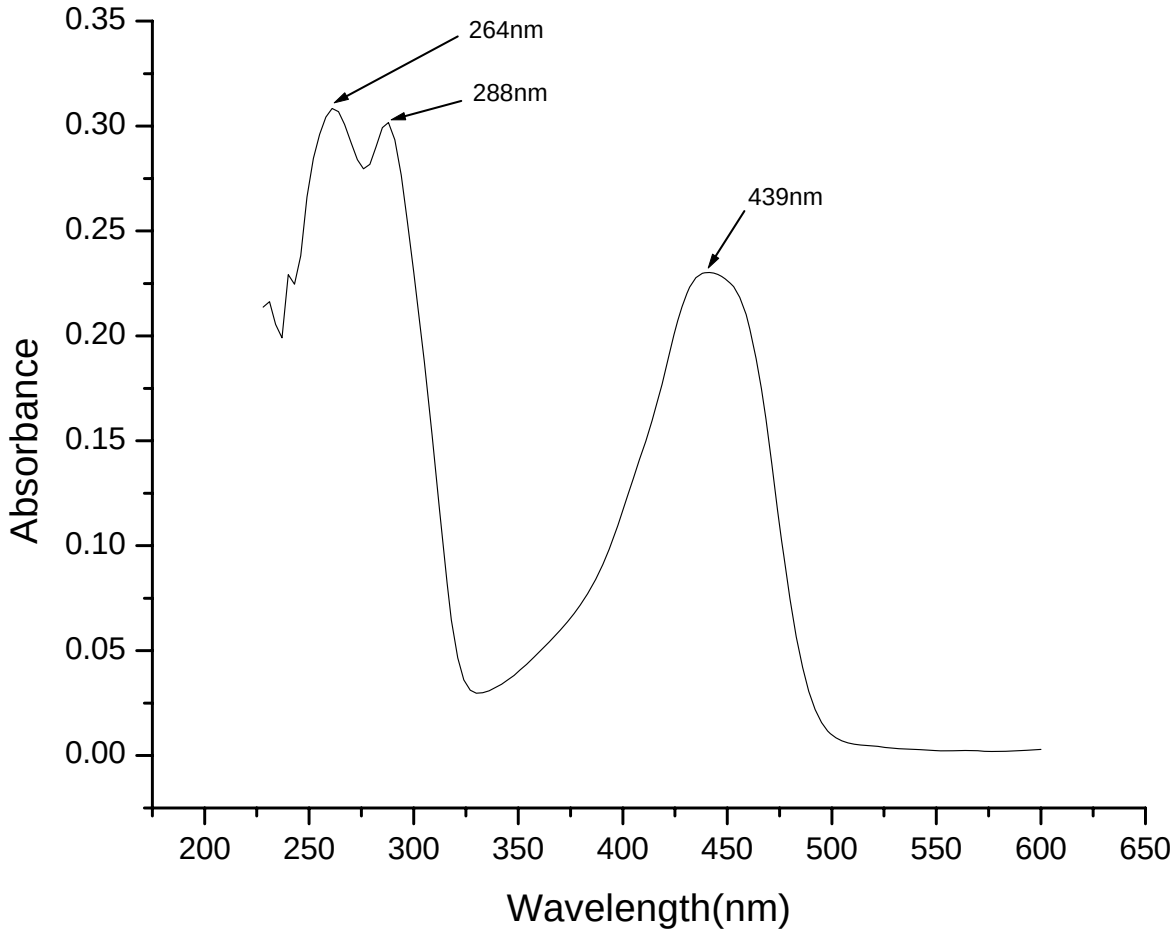
13. Appendixes

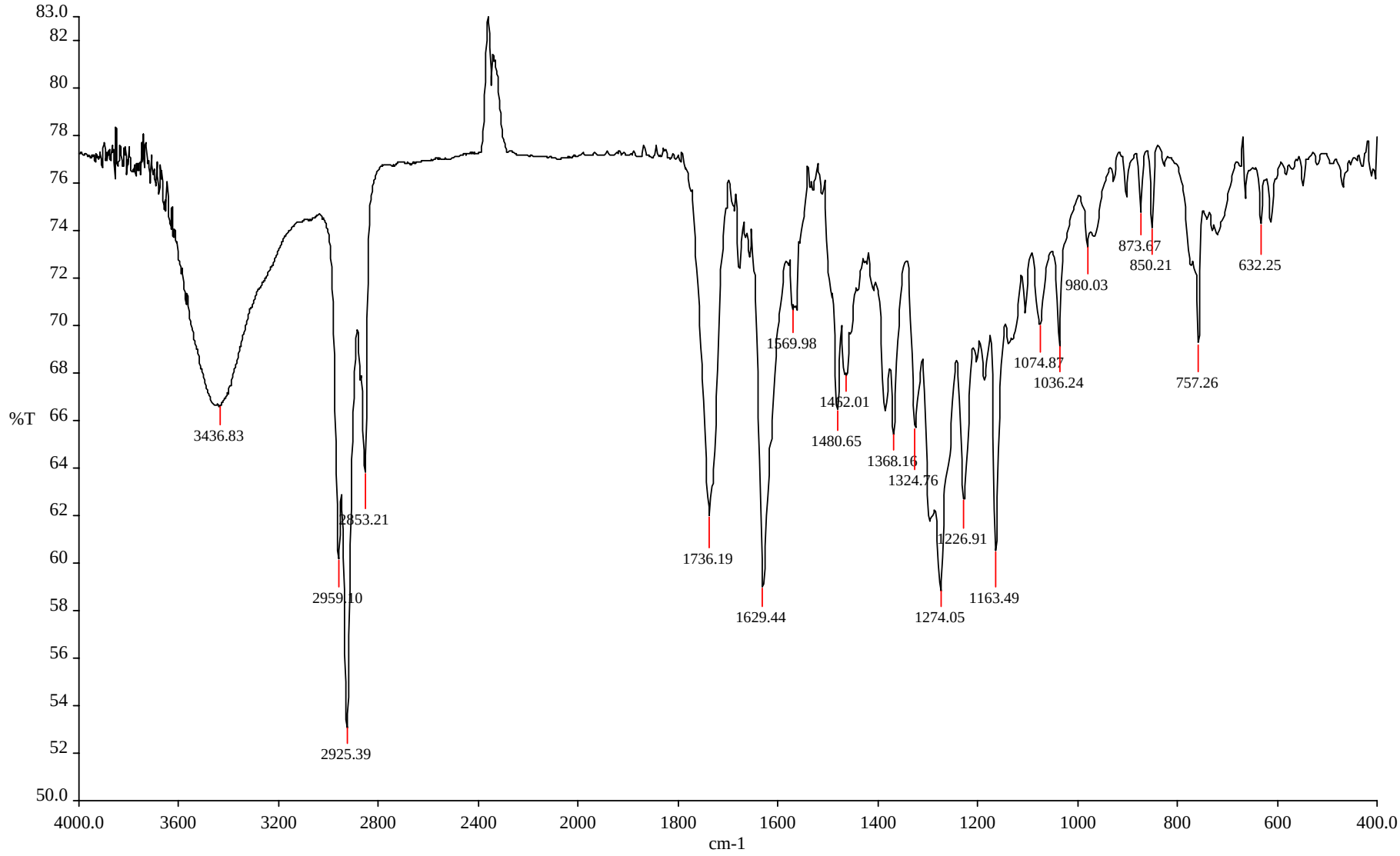
Appendix 1 . ^{1}H NMR Spectrum for Physcion (6) in $CDCl_3$



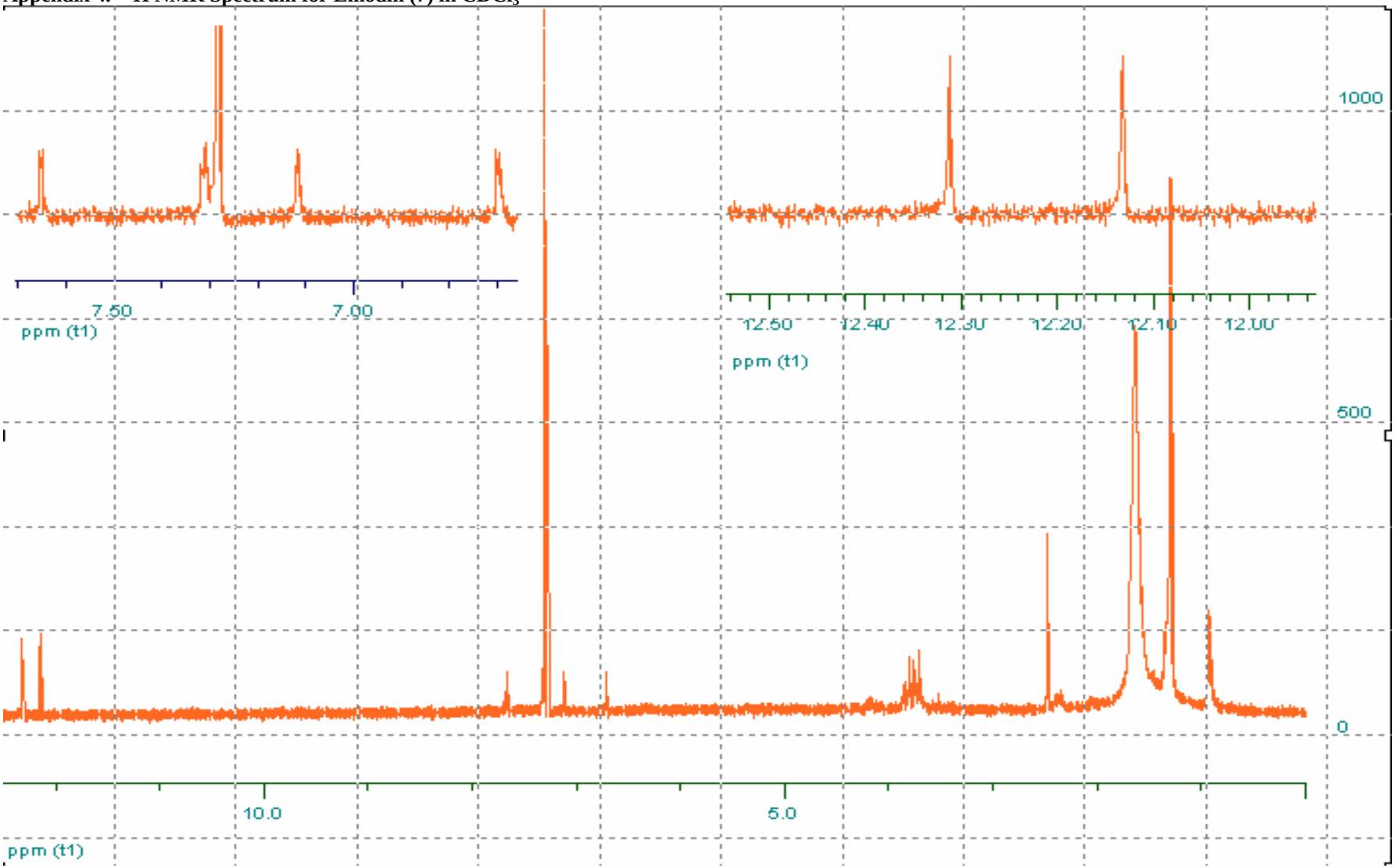
Appendix 2. UV Spectrum in $CDCl_3$ for Physcion compound (6)



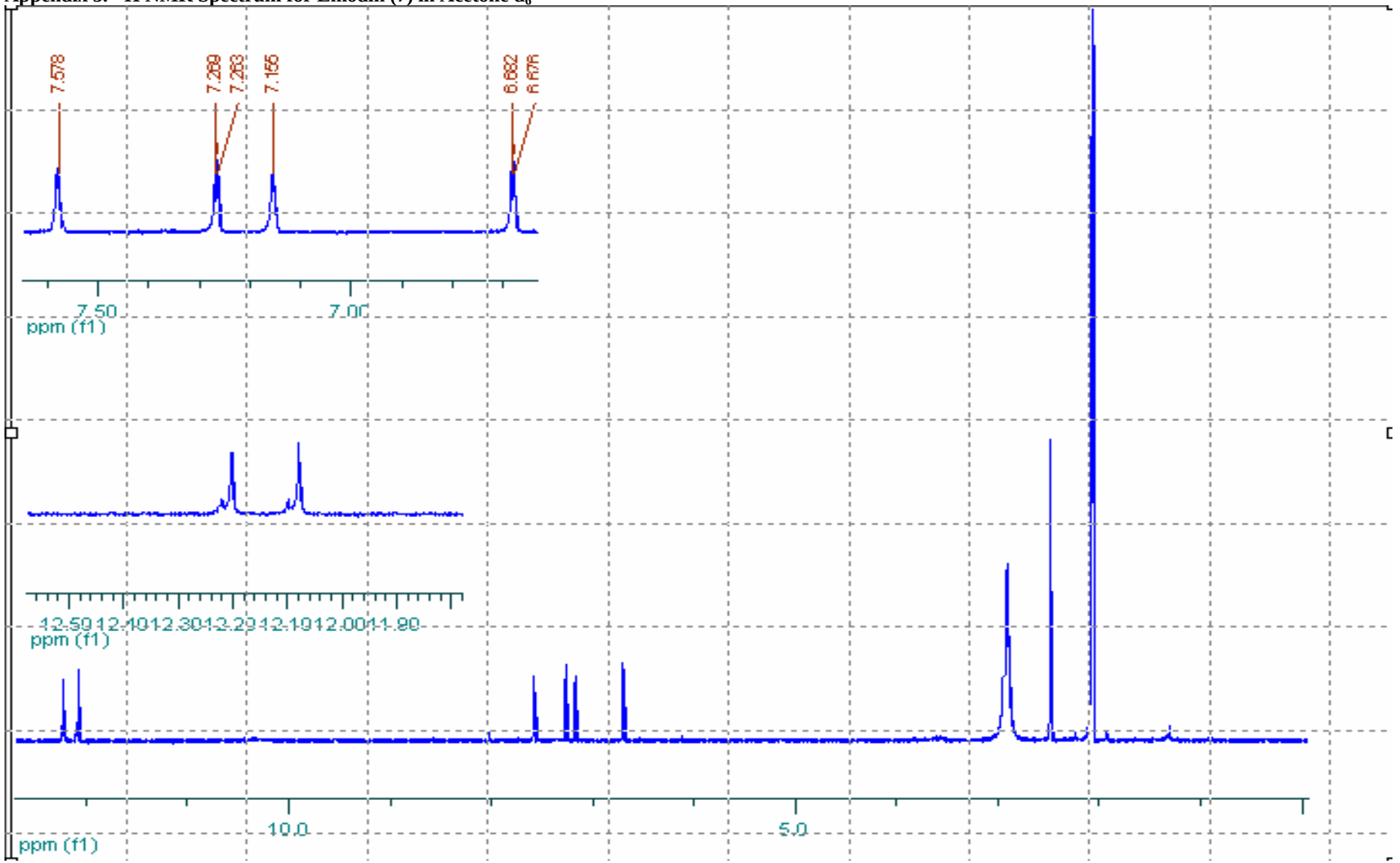
Appendix 3. IR Spectrum for Physcion (6) in KBr



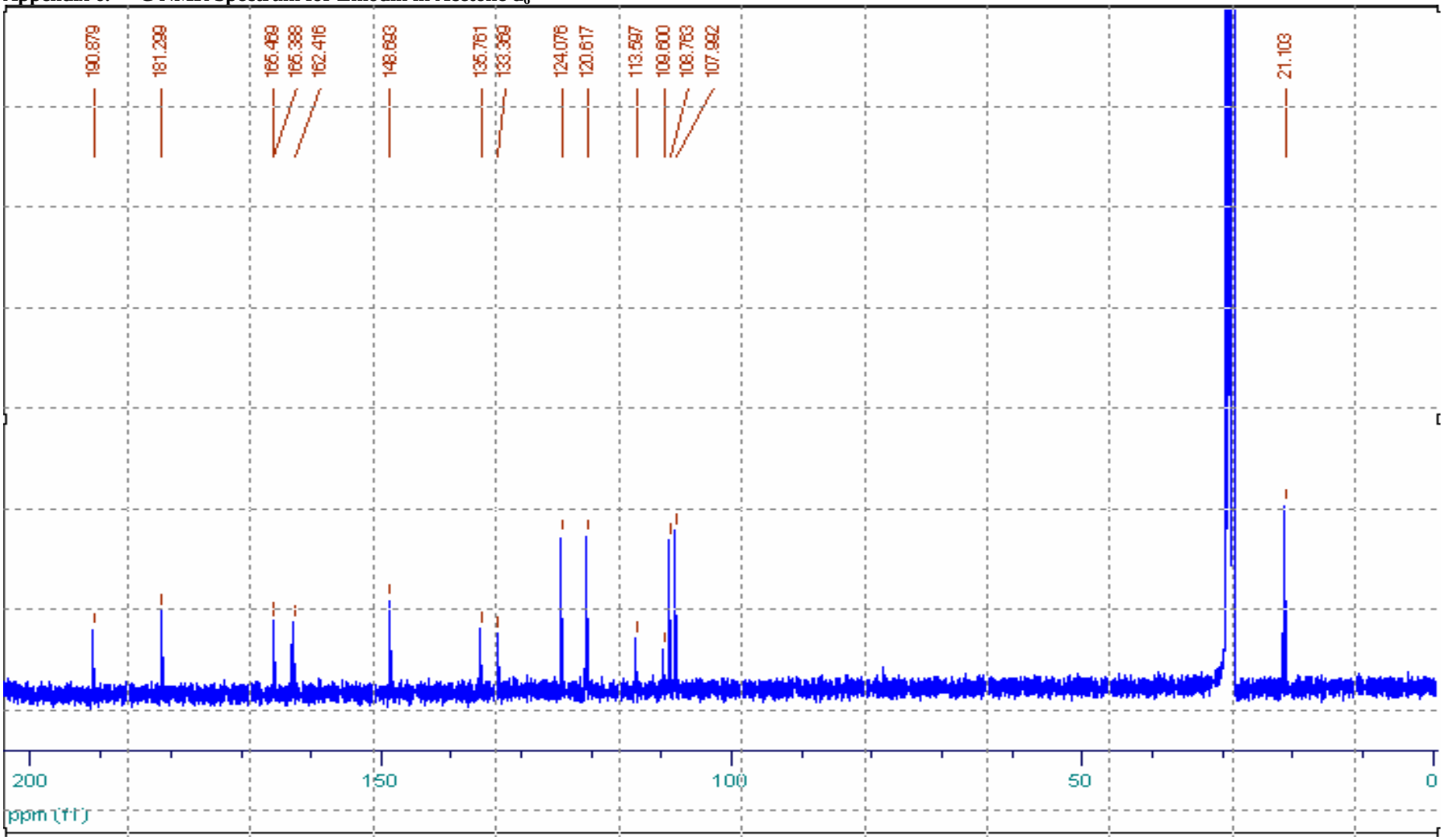
Appendix 4. ^{1}**H NMR Spectrum for Emodin (7) in** $CDCl_3$



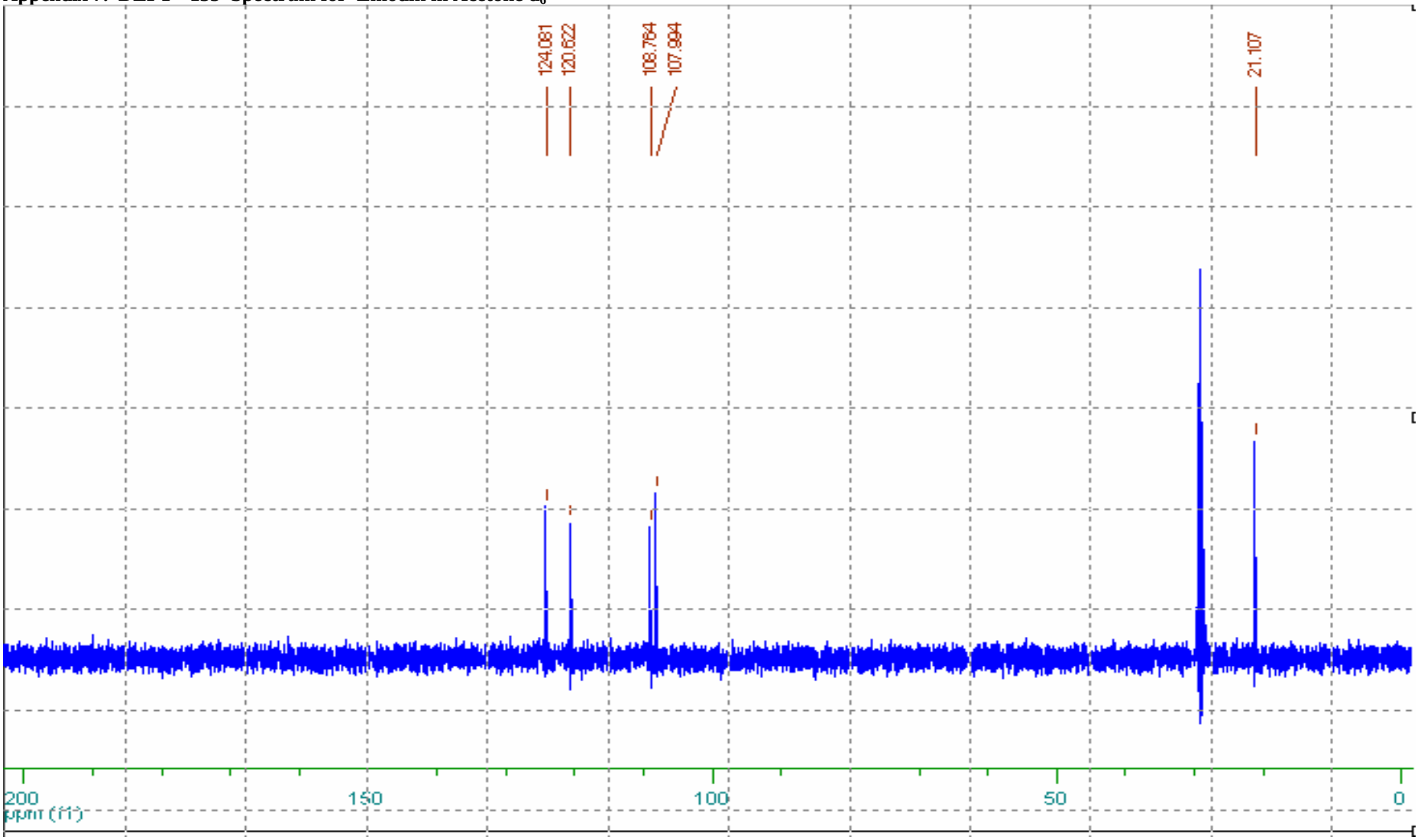
Appendix 5. ^{1}**H NMR Spectrum for Emodin (7) in Acetone d$_6$**



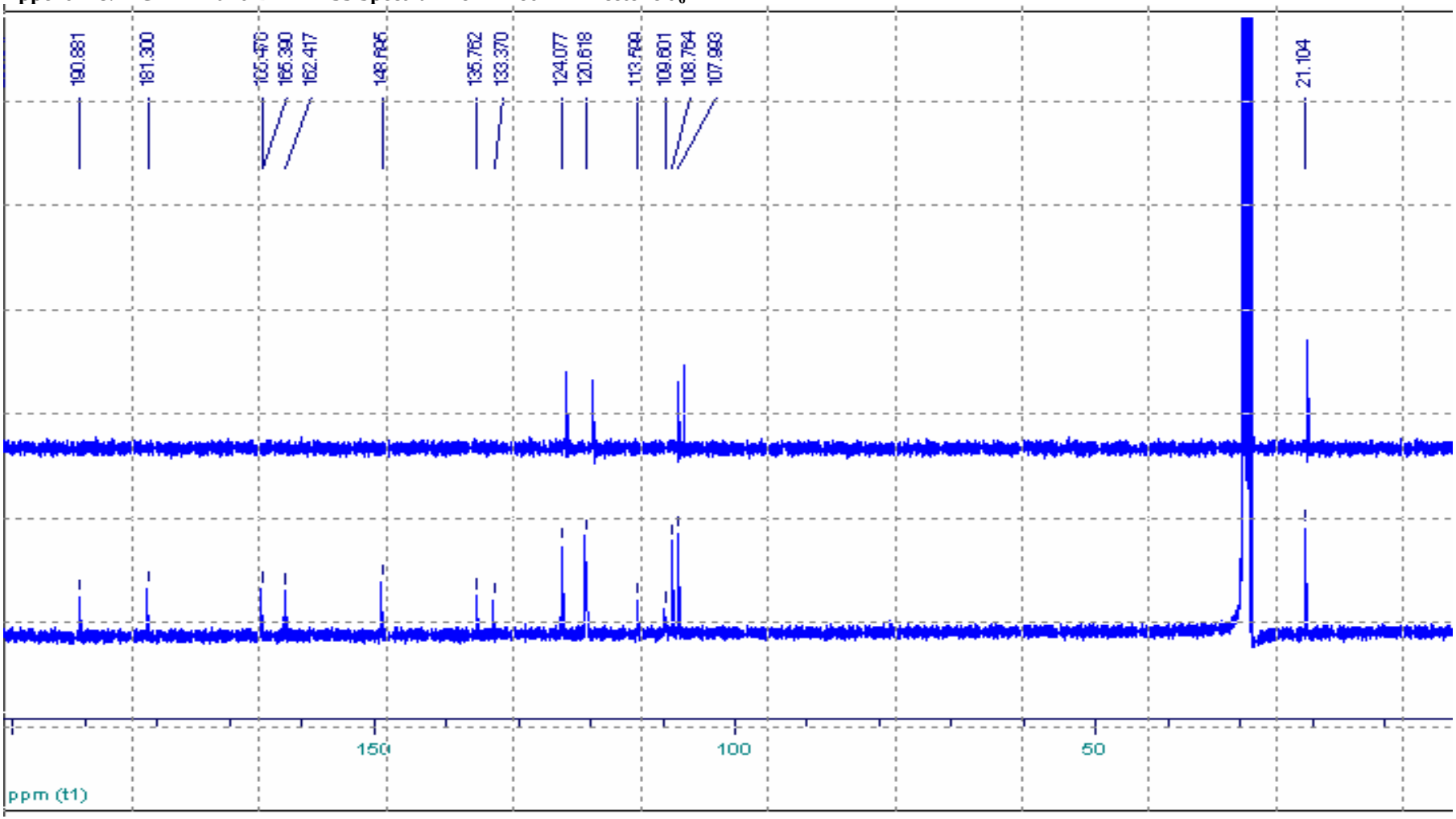
Appendix 6. ^{13}C NMR Spectrum for Emodin in Acetone d_6



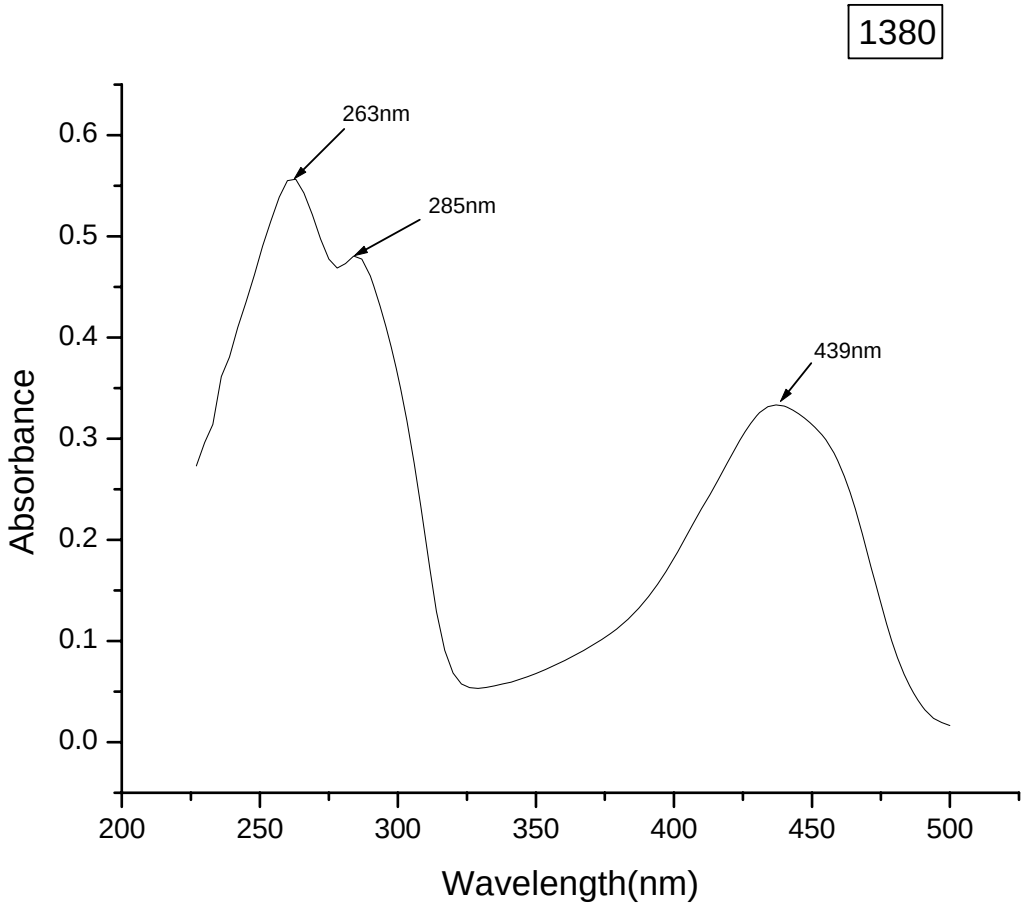
Appendix 7. DEPT- 135 Spectrum for Emodin in Acetone d_6



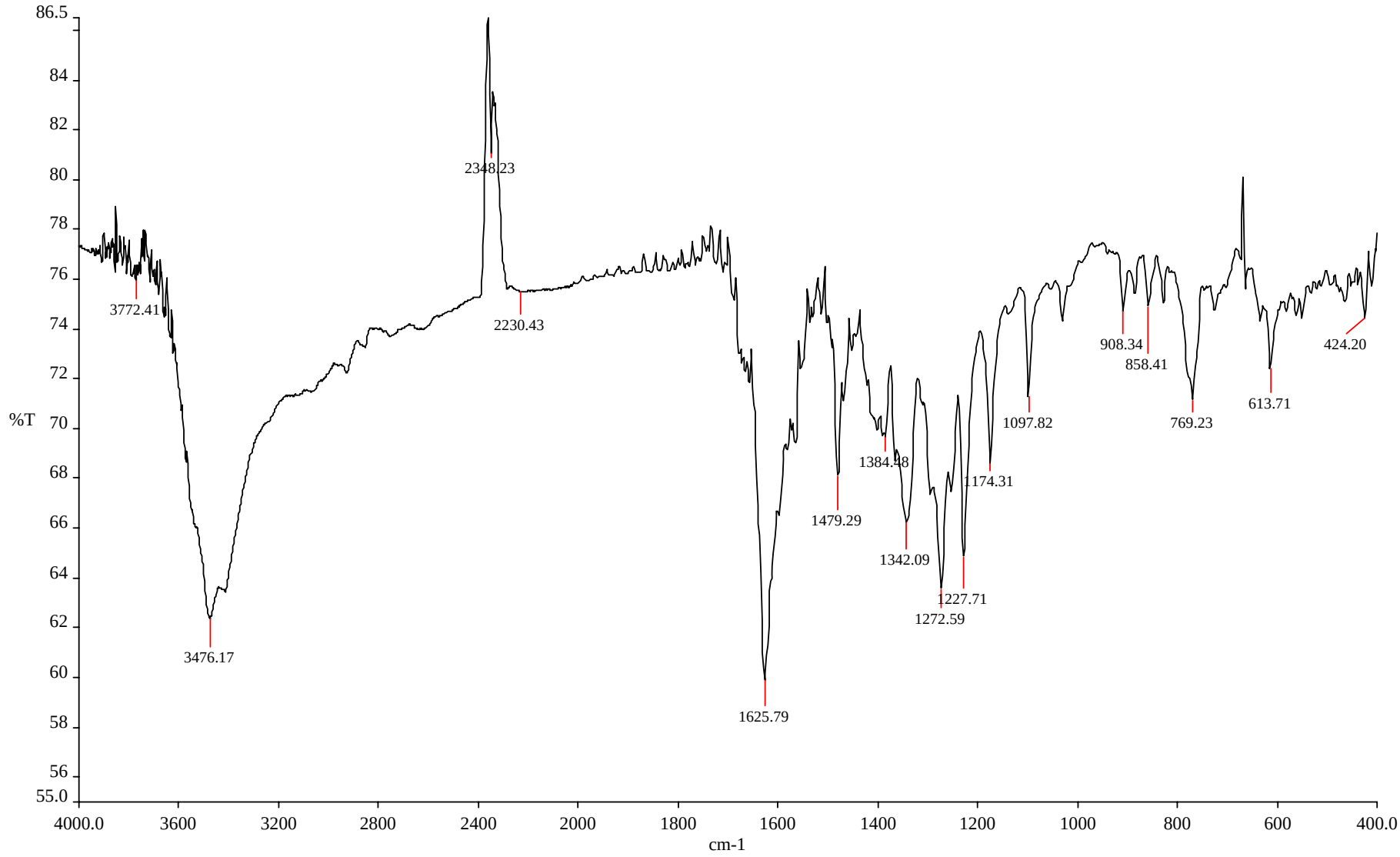
Appendix 8. ^{13}C NMR and DEPT-135 Spectrum for Emodin in Acetone d_6



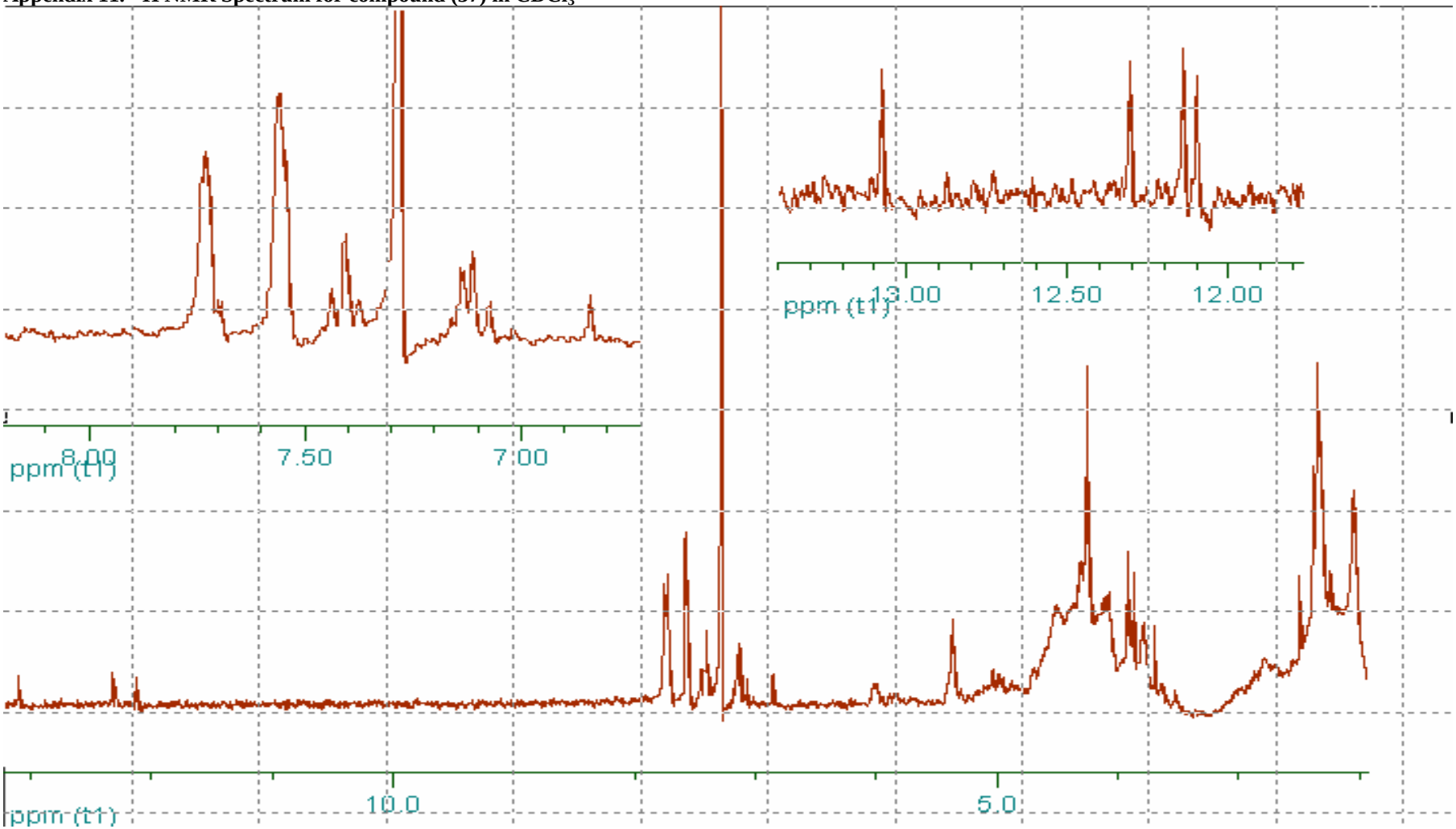
Appendix 9. UV Spectrum in $CDCl_3$ for Emodin (6)



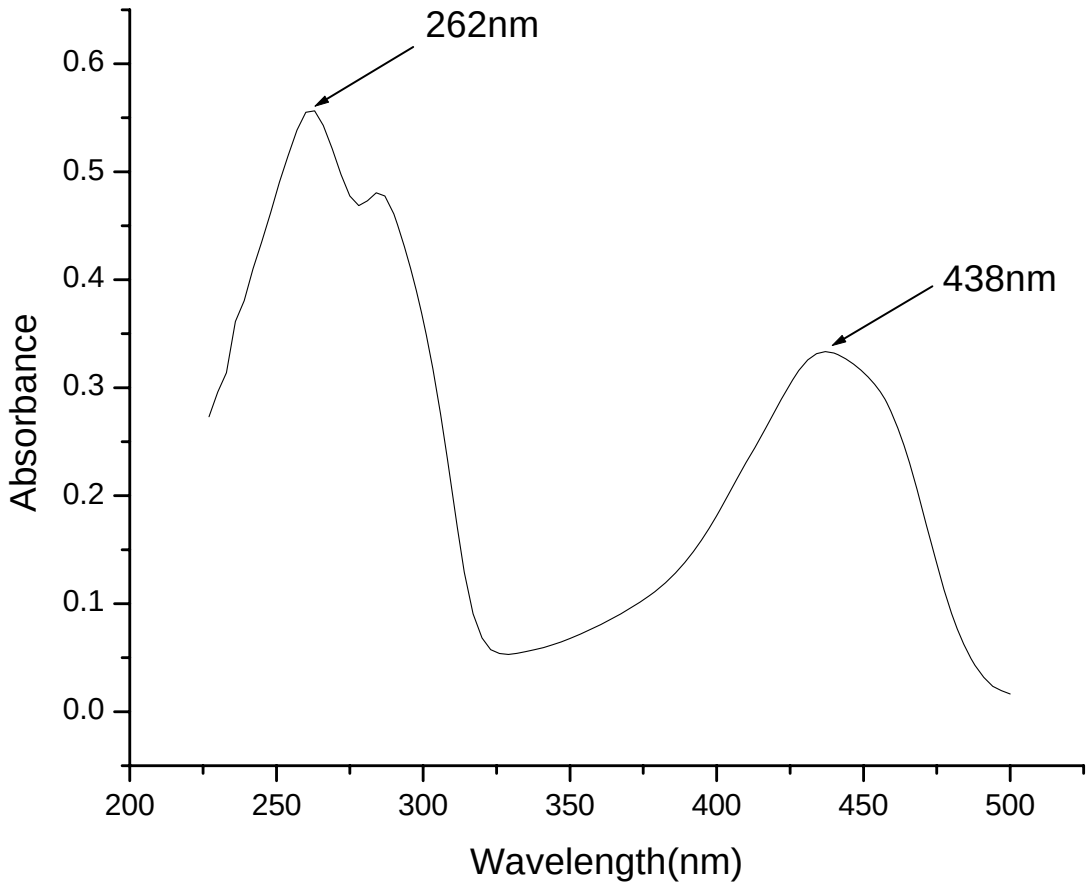
Appendix 10. IR Spectrum for Emodin (7) in KBr



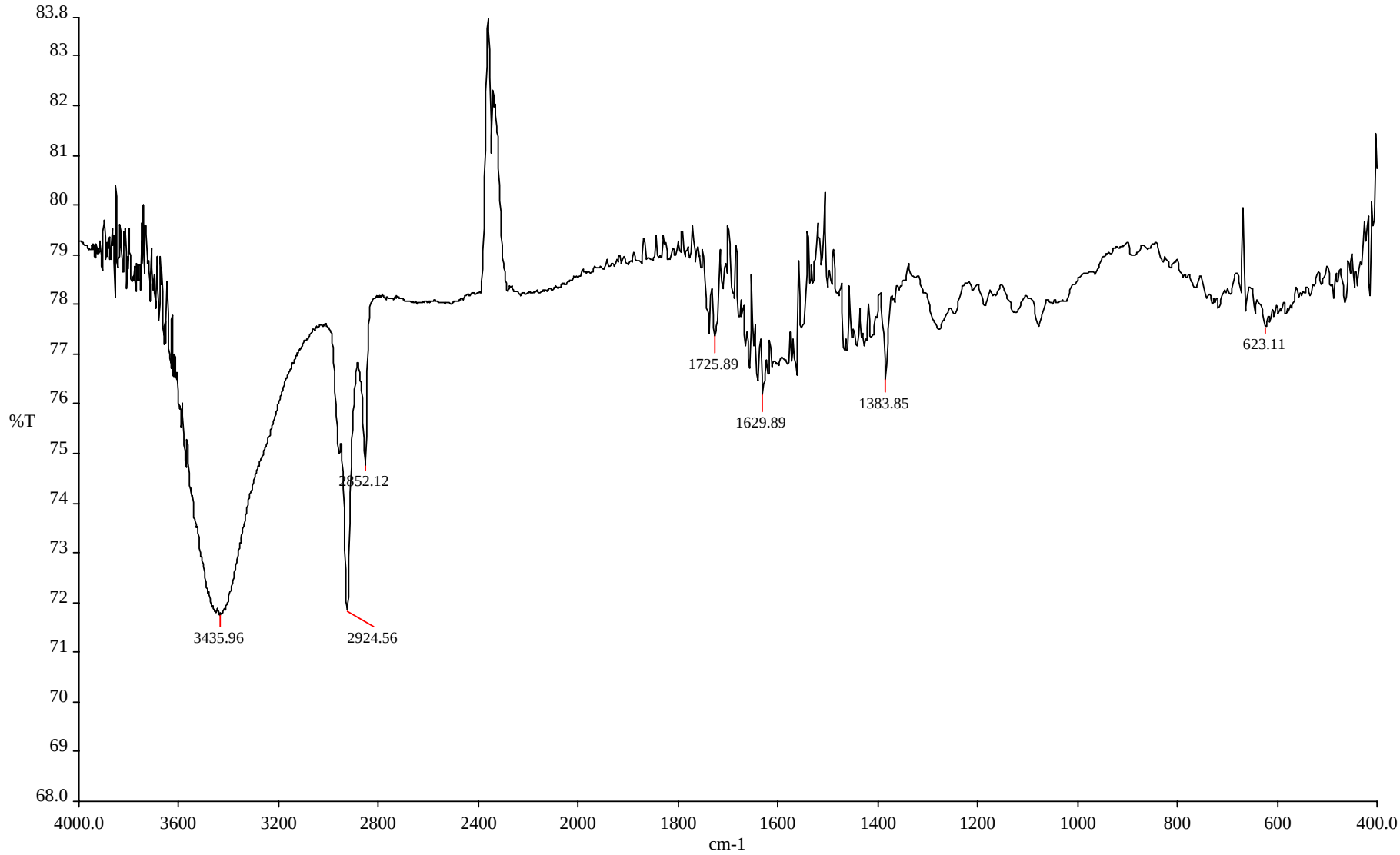
Appendix 11. ^{1}H **NMR Spectrum for compound (37) in** $CDCl_3$



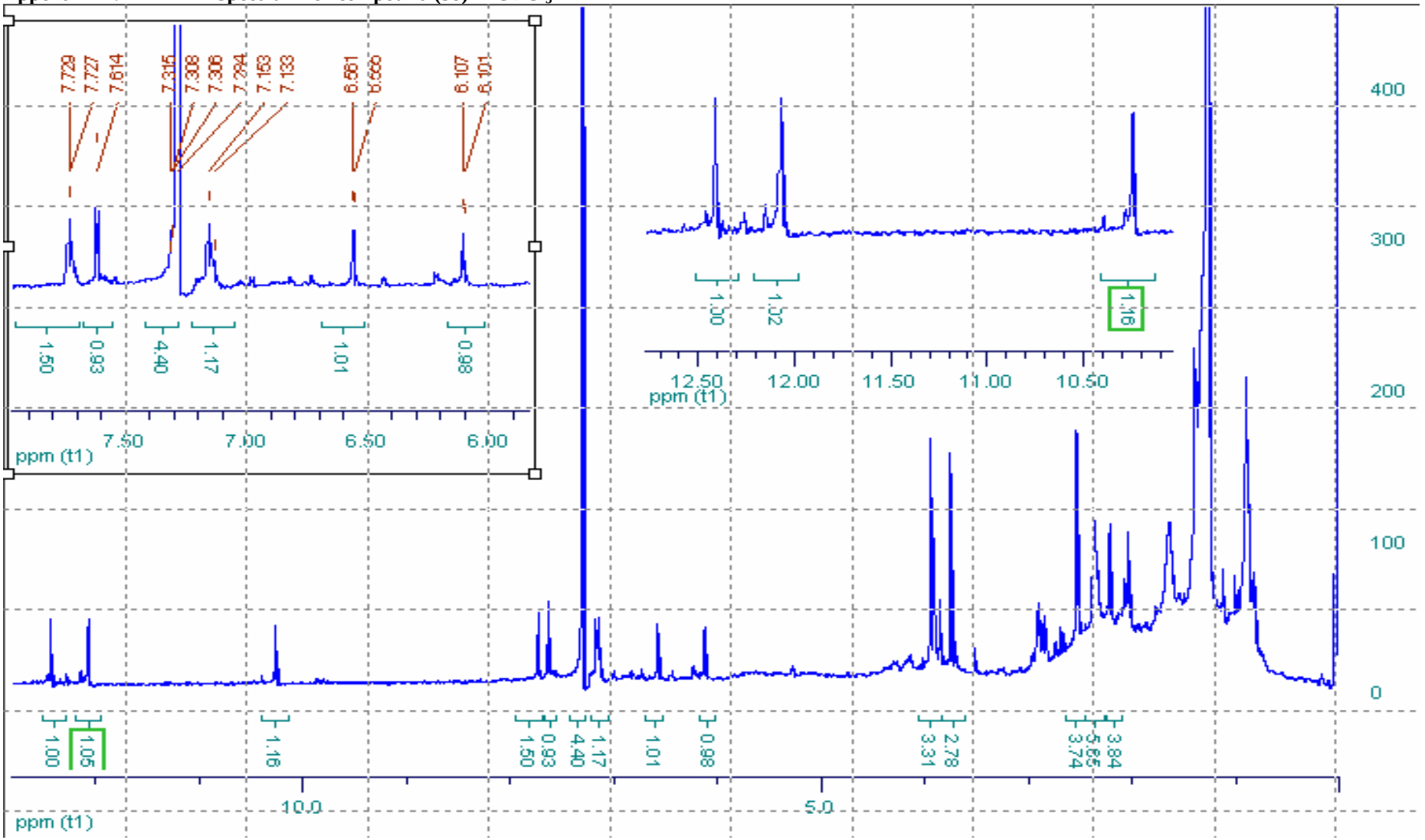
Appendix 12. UV Spectrum in $CDCl_3$ for compound (37)



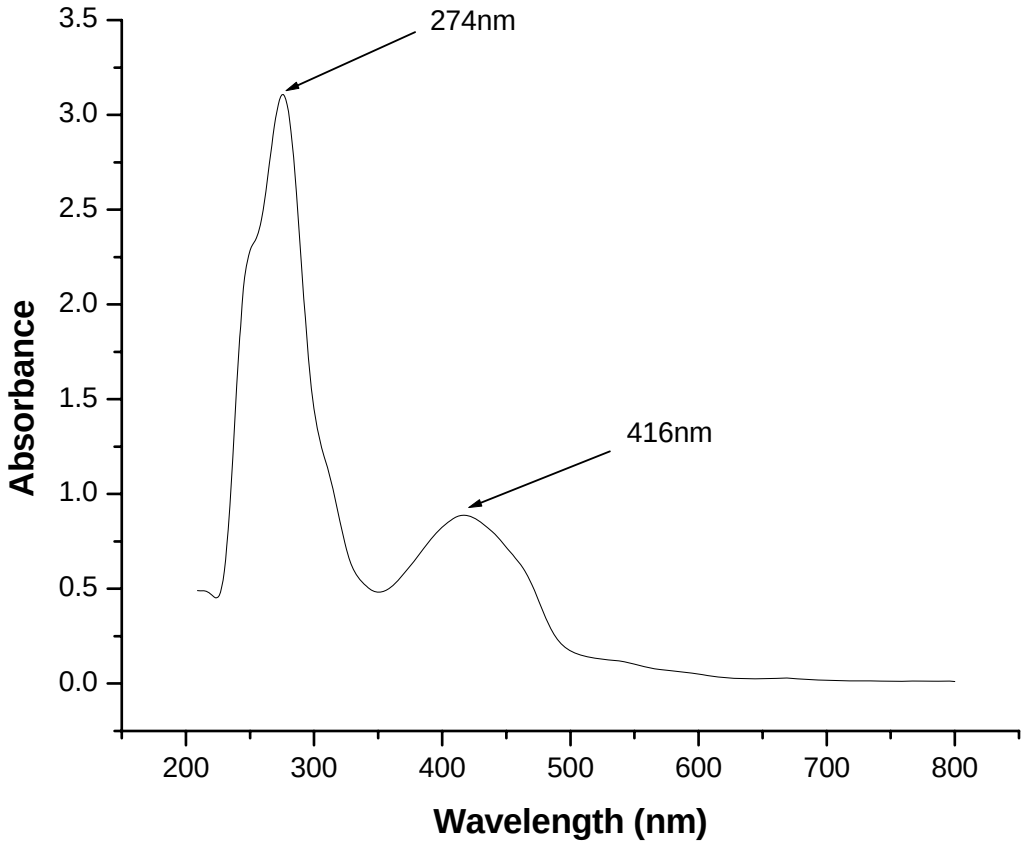
Appendix 13. IR Spectrum for compound (37) in KBr



Appendix 14. ^{1}H NMR Spectrum for compound (38) in $CDCl_3$



Appendix 15. UV Spectrum in $CDCl_3$ for compound (38)



Appendix 16. IR Spectrum for compound (38) in KBr