ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

PHYTOCHEMICAL INVESTIGATIONS ON THE FLOWERS OF *SENNA DIDYMOBOTRYA*

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JULY, 2005

PHYTOCHEMICAL INVESTIGATIONS ON THE FLOWERS OF *SENNA DIDYMOBOTRYA*

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BY
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petersiana, S. septemtrionalis, S. singueana, S. baccarinii, S. occidentalis, S. sophera, S. obtusifolia, S. siamea, s. ellisiae, S. longiracemosa, S. ruspolii, S. didymobotrya, S. truncata, S. italica, S. holosericea, S. multiglandulosa, S. alexanderia and S. bicapsularis.

People living in the villages of Africa, Asia and other parts of the developing world are forced to resort to traditional practitioners and to use traditional medicine for the continued maintenance of their health and to alleviate their diverse sufferings.[6] In this context, several species of *Senna*, having important medicinal properties, are also used as vital resource. They are mostly used in the treatment of sexually transmitted diseases (e.g gonorrhea), skin diseases and are sources of the well known *Senna* purgative. Some of them are also useful as appetizer.[7] Many *Senna* species are found to possess insecticidal properties and some exhibit antibiotic properties. *Senna* species are in general known for their use as cathartics in which this property is attributed to the presence of anthraquinones.[8]

Nature's abundant renewable supply of plants could be a great source of drugs, which can be complimentary to modern medicine. Systematic scientific investigations have resulted in identification of a growing number of active constituents many of which are routinely used in modern medicine. These include reserpine for the treatment of Cardiac arrhythmias, vincamine as a vasodilator, vinblastine and vincristine as anti-tumour agents.[9]

The effect of quercetin (common in senna species) on the infectivity and replication of HSV-1 (herpes simplex virus type-1), polio virus type-1, para influenza virus type-3 and respiratory syncytial virus (RSV) has been studied in cell structure and observed that quercetin caused a concentration-dependent reduction in the infectivity of each virus and, in addition, that intracellular replication of virus was reduced.[6]

It is believed that anthracene derivatives are highly active as anthrone glycosides, less active as free anthrones and much less active as free anthraquinones. Activity increases with the presence and position of the phenolic groups.[11] Pharmacological activity of both the free anthraquinones and the glycosides are being induced through their bioconversion into anthranols.[12] Further more, these pharmacologically important anthranols are believed to arise from the reduction of anthraquinones by intestinal micro flora.[12,13]

The importance of *senna* species as a drug is compiled in Table-1 below.

Table 1.Medicinal uses of some Senna species.

plant name	Plant part used	Purpose/Remedy for	Ref.
S. didymobotrya	leaf, root, stem bark	antimalarial, purgative, gonorrhea, cattle skin diseases, backaches, against ringworm, appetizer, antidote	7
S. petersiana	root, leaf,	gonorrhoea, haematuria, sterility, purgative, stomachache, anthelmintic, skin disease, coughs, syphilis	7
S. occidentalis	root, leaf,	abdominal pain, snake-bite, against round worm, oedema, fevers, malaria, antidote,stomachache, kidney troubles, pain-killer	7,10
S. singueana	root, bark, leaf	gonorrhoea,heartburn, purgative, stomach troubles, antibiotic	7,29

Table 1. cont.

S. sophera	seed, root	laxative, skin disease, acute bronchiate	9
S. italica	leaves, roots	laxative, gonorrhea	7, 10
S. septemtrionalis	Leaves, fruits	promote menstruation, purgative	7
S. alexandrina	root, bark, leaves	purgative	7, 10
S. obtusifolia	upper parts of the plant	stomach trouble, quicken birth, antibiotic	7, 10
S. bicapsularis	roots	stomach complaints in children	7

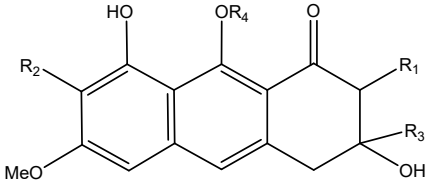
3. Secondary Metabolites Isolated from the Genus *Senna*

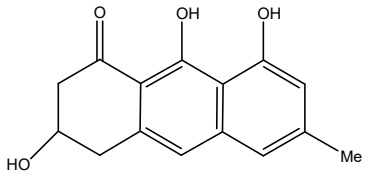
Phytochemical studies on the genus *Senna* has led to the isolation and characterization of different classes of secondary metabolites. Anthraquinones are the most abundant class of metabolites reported with chrysophanol, physcion and emodin as the common anthraquinones. Alkaloids, flavonoids, xanthones, tannins and terpenoids are also reported to occur in this genus. A search through the Dictionary of Natural Products shows that there are 409 compounds isolated from the former genus *Cassia* (*Cassia, Chamaecrista and Senna*). [14]

3.1. Preanthraquinones

Five preanthraquinones have been reported from various *senna* species.

It is assumed that the preanthraquinones are the biosynthetic precursors to the corresponding anthraquinones.[15] They include torosachrysone (**1**) from *S. Singueana* root bark [16] in vitro cultures of S. didymobotrya [17] and the leaves of *S. didymobotrya*.[18] Torosachrysone was first isolated from the seeds of *S.torosa*.[19] 7-Methyl torosachrysone (**2**) occurs in the tissue cultures of *S. occidentalis*[20]; germitorosone (**3**) and germitorosone -9 - methyl ether (**4**) in S. torosa [21] and tissue cultures of S. occidentalis [20]; and germichrysone (**5**) in the root bark of S. singueana [16] and in vitro cultures of *S. didymobotrya*. [17]





5. Germichrysone

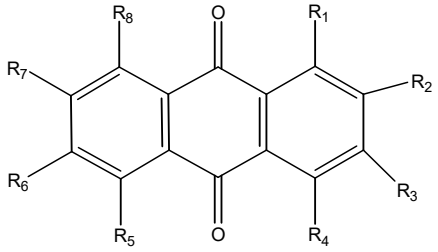
	R_1	R_2	R_3	R_4
1. Torosachrysone	H	H	H	H
2. 7- Methyl torosachrysone	H	Me	H	H
3. Germitorosone	OH	Me	H	H
4. Germitorosone - 9 - methyl ether	OH	Me	Me	Me

3.2. **Anthraquinones**

Anthraquinones constitute an important class of compounds with important biological properties. These compounds are also one of the most well known naturally occurring pigments.

Anthraquinones and related compounds in higher plants are located in all parts of the higher plant, including root, heart wood, bark, leaves, seeds and often occur as glycosides. [9]

More than 25 *senna* species studied are known to contain anthraquinones. The identified anthraquinones, with the anthracene derivative skeleton shown, are very well documented as dihydroxy, trihydroxy, pentahydroxy and hexahydroxy derivatives.[22,23]



6. Anthracene Derivative Skeleton

A number of dimeric anthraquinones have been isolated and all the dimeric anthraquinones so far reported have only C-C linkage in between the two units . No bianthraquinone has been reported with a C-O-C inter anthraquinoid linkage.
Anthraquinones and bianthraquinones isolated so far from Ethiopian senna species are compiled in Tables **2** and **3** respectively.

Table3. Bianthraquinones isolated from Ethioian *Senna* species

Compound No.	Name of the compound	Source	References
34	Floribundone-1	*S. floribunda*	[63,65]
35	Floribundone-2	*S. sophera* *S. multigladulosa* *S. floribunda*	[5,59,52] 63,64,65 67,68]
36	Totosaol-III	*S. sophera*	[63]
37	Torosanin-9,10,-quinone(7,5-Physcion-torosachrysone)	*S. sophera* *S. floribunda* *S. multiglandulosa*	[5, 59, 63, 65]
38	Phlegmacine A_2 and B_2	*S. sophera*	[60, 61, 62]
39	Anydrophlegmacine-9,10-quinone A_2 and B_2	*S. sophera* *S. floribunda* *S. multiglandulosa*	[59, 52, 60, 61, 67]
40	Anhydrophlegmacine B_2	*S. sophera*	[60, 61]
41	Physcion-10,10'-bianthrone	*S. sophera* *S. siamea*	[23, 52, 60]

Table 3 continued…

42	Torosanin	*S. sophera*	[61]
43	Isosengulone	*S. sophera* *S. multiglandulosa*	[52, 68]
44	Sengulone	*S. sophera* *S. multiglandulosa*	[52, 68]
45	7,5'- physcion-fallacinol	*S. floribunda*	[5]
46	Cassiamin A(2,2'-chrysophanol-emodin)	*S. siamea*	[22, 23, 69]
47	Cassiamin B(2,2'- biemodin)	*S. siamea*	[22, 23, 69]
48	Cassiamin C (2,2'-bichrysophanol)	*S. siamea* *S. occidentalis*	[22, 23, 69]
49	Siameanin (4,4'-bichrysophanol)	*S. occidentalis*	[22]
50	10-(physcion-7'-y1)-10-hydroxychrysophanol-9-anthrone	*S. didymobotrya*	[48,66]
51.	10,10'-bichrysophanol	*S. siamea* *S. longiracemosa*	[50, 58]
52	Presengulone	*S. sophera*	[52]
53	Siameadin (4,4'-chrysophanol-emodin bianthrone	*S. occidentalis*	[23]

Table 3 continued…

54	Palmidin D (10, 10'-chrysophanol - physcion bianthrone	*S. longiracemosa* *S. occidentalis* *S. alexandrina*	[23, 49, 58]
55	Torosaol- I	*S. sophera*	[62]
56	Torosaol- II	*S. sophera*	[62]
57	Singuealone- I	*S. sophera* *S. singueana*	[62]
58	Singuealone- II	S. singueana	[63]
59	Palmidin A (10, 10'-aloe-emodin-emodin bianthrone	*S. alexandrina*	[49]
60	Aloe-emodin-10, 10'-bianthrone	*S .alexandrina*	[49]
61	10, 10'-Chrysophanol-isophyscion bianthrone	*S. longiracemosa*	[58]
62	10- (Chrysophanol-7'-yl)-10-hydroxychrysophanol	*S. longiracemosa*	[58]
63	Rheidin B (10,10'- rhein-chrysophanol bianthrone	*S. alexandrina*	[49]
64	Palmidin C (10,10'-rhein-chrysophanol bianthrone)	*S. alexandrina*	[49]

Table 3 continued…

65	Palmidin B (aloe- emodin-chrysophanol bianthrone)	*S. alexandrina*	[49]
66	10,10'-Emodin bianthrone	*S. alexandrina*	[49]
67	Occidentalol-I	*S. occidentalis*	[70]
68	Occidentalol-II	*S. occidentalis*	[70]
69	5,10-Dihydroxy-2-methyl-9-(physcion-7'-yl)-1,4-anthraquinone	*S. didymobotrya*	[48,66]
70	Rheidin A (rhein-emodin bianthrone)	*S. alexandrina*	[49]
71	Rhein- 10, 10'- bianthrone	*S. alexandrina*	[49]
72	Rhein- aloe- emodin- 10, 10'-bianthrone	*S. alexandrina*	[49]
73	10, 10'- Biisophyscion	*S. longiracemosa*	[58]

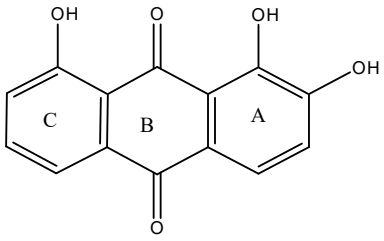
3.2.1. Biosynthesis of Anthraquinones

The structure and substitution pattern of anthraquinones generally reflect two modes of biosynthetic pathways. The acetate-malonate pathway and the shikimate-mevalonate pathway. [29-31]

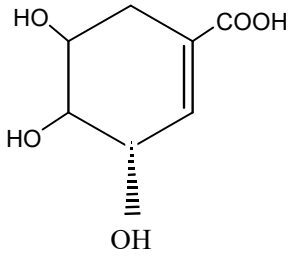
Many common anthraquinones found in micro-organisms, lower animals and plants are mainly of polyketide origin. They are categorized depending on how one or both A and C rings bear particular substituients such as OH, OCH_3, CH_3, etc. It appears that, at least in higher plants, those anthraquinones with substituents on both A and C rings are derived via the acetate-malonate pathway. These compounds of

polyketide origin, usually with a C-15 skeleton, are exemplified by emodin (**10**). Those compounds with only one ring substituted, such as alizarin(**7**) derive from shikimate and mevalonate route in some plant families.

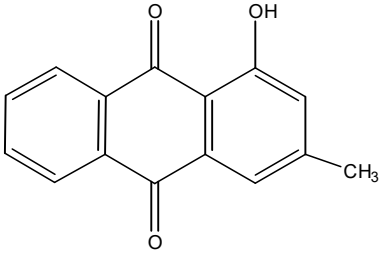
Shikimic acid (**8**) derived from phosphoenol pyruvic acid and erythro-4- phosphate is incorporated in to alizarin together with the three central carbon of α- ketoglutaric acid, an intermediate of tricarboxylic cycle. However, the situation in lower organisms is not so clear-cut. Since pachybasin(**9**) which ought to be derived from shikimate, does in fact arise via the acetate-malonate path way.[32]



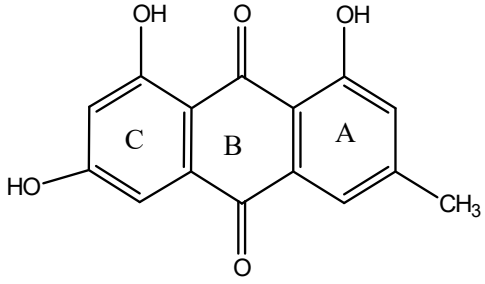
7. Alizarin



8. Shikimic acid



9. Pachybasin



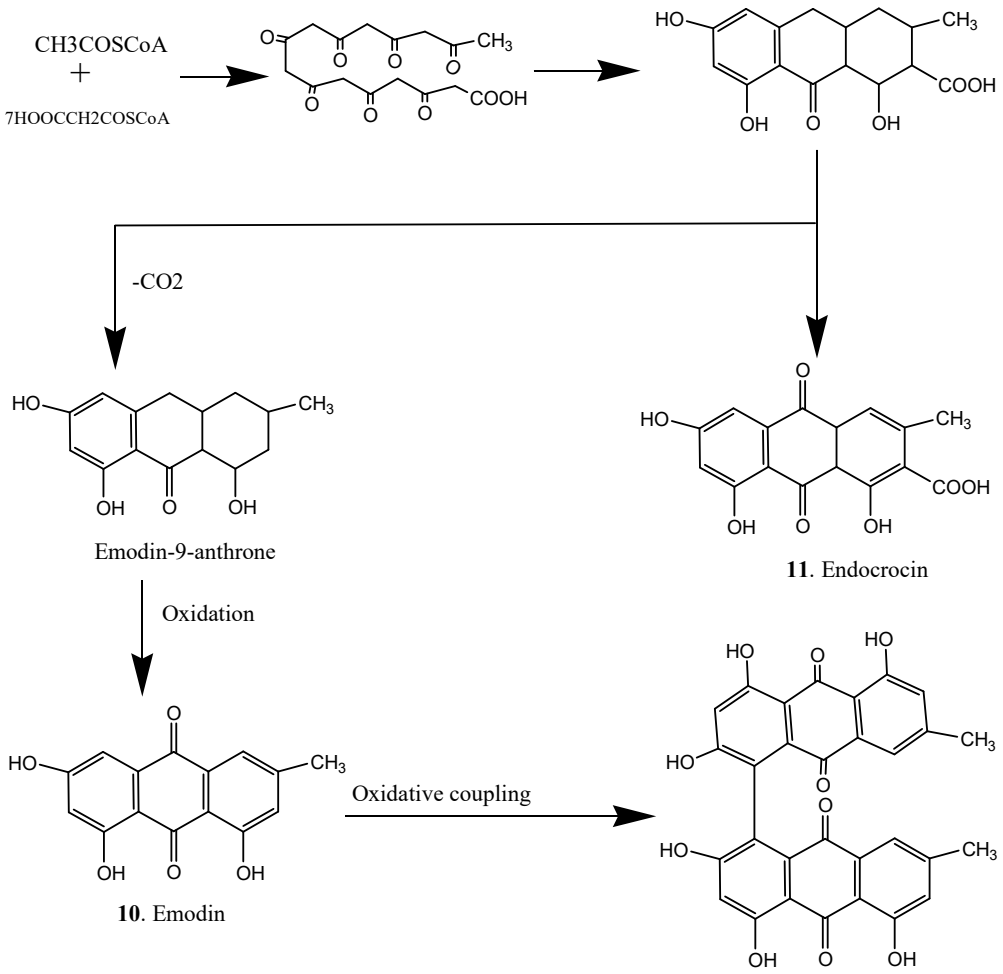
10. Emodin

Most of the acetate derived anthraquinones examined so far are formed by cyclization of an octaketide chain. Other steps in the biosynthesis of these anthraquinones involve decarboxylation, reduction and aromatization of the cyclized polyketide.

Anthrones are biosynthetic intermediates which lead to anthraquinone structures and the oxidative coupling of anthraquinones leads to dimeric anthraquinones. The biosynthetic pathway which leads to emodin (**10**) and endocrocin(**11**) is given in Scheme**1**.

Anthraquinones of the emodin type take their start at carbon oxygen skeleton generated from joining of acetate units, in head-to tail linkage, which then undergo subsequent structural modifications that may include the introduction and removal of oxygen, alkylation (notably with methyl), glycosidation and dimerization.

Anthraquinones can arise from either a single polyketide chain or from more than one polyketide chain. The active form of the first C_2 unit is acetyl coA, while malonyl COA, formed by carboxylation of acetyl COA, is used for subsequent C_2 units. This utilization of two different units by the enzyme accounts for the different distribution of radioactivity in polyketide molecules obtained after incorporation of labeled malonic acid.



Scheme 1: Biosynthesis by the polyketide pathway

The co-occurrence of torosachrysone, physion and physcion anthrone with phlegmacines, anhydrophlegmacin-9-10- quinone and totrosanis in C. torosa [24,25] suggests that, similar biogenetic route, (which involves the conversion of preanthraquinoues to anthraquinones) may be followed in higher plants too.

Experimental evidence indicated [26] that in the seedlings of C. torosa, germichrysone is not derived from anthraquinone, but it is a product of denovo biosynthesis. Thus, it is possible to infer that, in plants the reduction of anthraquinones to pre-anthraquinones is least likely to occur.

3.2.2. Spectral properties

3.2.2.1. IR Spectroscopy

The appearance of more than one hydroxide band between 3600 and 3150 cm^{-1} indicates the presence of more than one such hydroxyl group attached to the anthraquinone nucleus, although the reverse does not always hold.

In anthraquinones with no –hydroxyl groups the single carbonyl frequency occurs between 1678 and 1653cm^{-1}. Chelated quinones can be recognized by their displaced carbonyl absorption (1616-1592cm^{-1}) together with the downward shift in hydroxyl frequency. The lowered frequency of the δ -hydroxyl group and the accompanying changes of intensity and frequency in the carbonyl region have been ascribed by Hilbert etal [33] as chelation and later modified as conjugated-chelation [34] where the strength of hydrogen bonding between the hydroxyl and the carbonyl group is enhanced by resonance with an ionic form having a conjugated system in which the donor acceptor properties of the chelating centers are increased.

Table 4. Carbonyl frequencies of hydroxyl anthraquinones in cm^{-1} [35]

Type of anthraquinone	Carbonyl frequencies
No alpha OH	1678 – 1653
1 OH	1675 – 1647,1637 - 1621
2 OH (1,4&1,5)	1645- 1608
2 OH (1,8)	1678 – 1661, 1626 – 1616
3 OH (1,4,5)	1616 - 1592

3.2.2.2. UV-Visible Spectroscopy

The UV absorption spectrum of anthraquinones is considered to originate from the benzenoid and quinone chromophoric system.The bands at 252 and 325 nm are associated with the benzenoid character of the molecule, and those at 262 and 272 nm are considered to orginate from the C=C bond of the quinone accompanied by weak quinoid absorption band at 405nm. These bands are still evident, more or less modified, in substituted anthraquinones.The effect of substitution (electron donating and with- drawing) on the UV-Vis spectra is discussed in the literature. [36-40]

3.2.2.3. NMR Spectroscopy

NMR spectroscopy has proved invaluable as a guide to structural determination. Chemical shifts, splitting patterns and coupling constants greatly aid in structural assignment and determination of orientation of substituents. In proton NMR, the alpha and beta protons of 9,10-anthraquinone give multiplets centered at 8.07 and 7.67ppm respectively and are modified by substitution. The hydroxyl protons on 1,4,5 and 8 positions are easily distinguished from other types of hydroxyl protons by their appearance at unusually low field resonance between 11 and 14 ppm or above. Chelation of hydroxyl groups with 9,10 keto groups accounts for the shift.

Steglich and Lösel [41] studied the chemical shift differences for the corresponding aromatic protons in the ^{1}H NMR spectra of pre- acetylated and pre trimethyl silylated anthraquinones and found the acylation shift data as useful parameters in

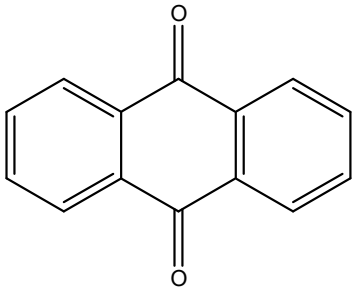
determining the positions of 0–alkyl or 0– glycosyl substituents in 1,8 dihydroxy antaraquinone derivatives. These findings are based on the well known acetylation shifts in phenols where ortho protons are shifted by – 0.25 ppm and para protons by -0.20 ppm whereas meta protons remain unaffected. The presence of many hydroxyl groups leads to an additive effect.

In ^{13}C NMR spectra of anthraquinones, carbon 9 and 10 are easily recognized by their resonance occurring between 180 and 185 ppm while carbons bearing hydroxyl groups appear at about 160 ppm. [42]

3.2.2.4. Mass spectroscopy

Anthraquinones generally show similar tendency to eliminate neutral carbon monoxide. In the mass spectra of anthraquinone (**12**), the most abundant ions are of mass 208 (M^+), 180 (M^+ -CO) and 152 (M^+ - 2CO).

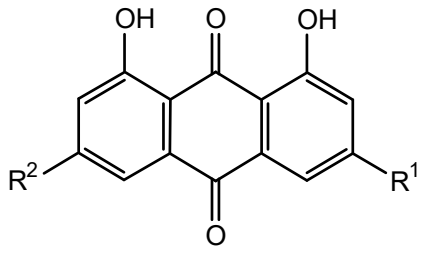
Accurate mass measurements established that these two latter ions were produced by successive loss of two neutral CO fragments. [43]



12. Anthraquinone

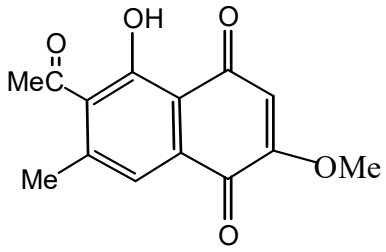
4. *Senna* didymobotrya

S. didymobotrya is a bushy shrub about 3-4 m high or occasionally a tree up to 6m high, leaves up to 0.5m long, common from 1450m to 2450m in good rainfall areas.[44] In Ethiopia, it is found in Wello, Welega, Shewa, Sidamo, & Arsi regions. [10]
Previous phytochemical investigation on the various parts of the plant including leaves, pods & stem barks were reported.
From the leaves, chrysophanol(**23**), physcion(**24**), aloe-emodin(**13**), fallacinol(**14**), rhein(**15**), parietenic acid(**16**), torosachrysone, kaempferol-3-rhamnoside and isoquerctin [45,46] were isolated.

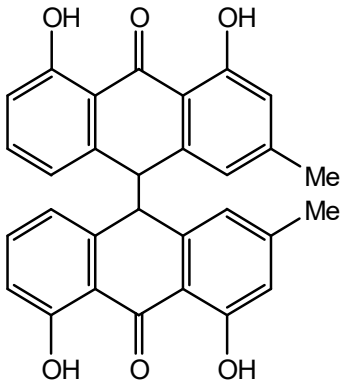


	R^1	R^2	
13.	CH_2OH	H	aloe-emodin
14.	CH_2OH	OCH_3	fallacinol
15.	COOH	H	rhein
16.	COOH	OCH_3	parietinic acid

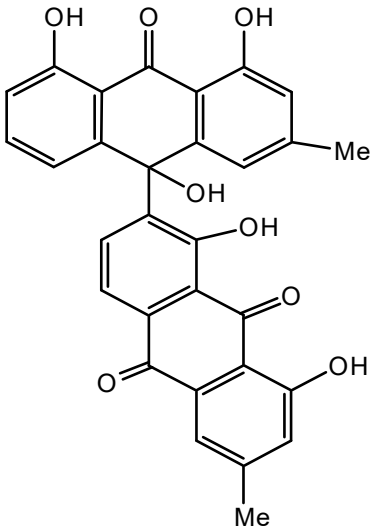
2-Methoxystypandrone(**17**), 10,10-bichrysophanol(**18**) 10- (chrysophanol –7'- yl)-10 hydroxy chrysophanol-9-anthrone(**19**) and fallacinol(**14**) along with the three common anthraquinones physcion(**24**), chrysophanol(**23**) and emodin(**10**) were reported from the stembarks. [47]



17. 2-Methoxystypandrone

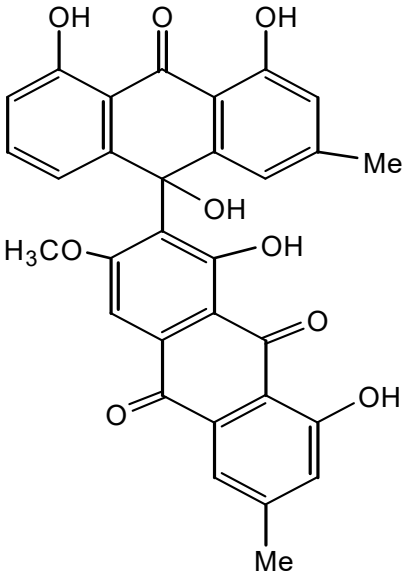


18. Chrysophanol-10-10'-bianthrone

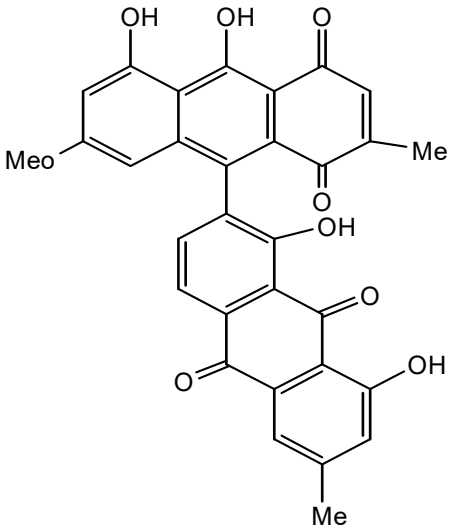


19. 10-(chrysophanol-7'-yl)-10-hydroxy-chrysophanol-9-anthrone

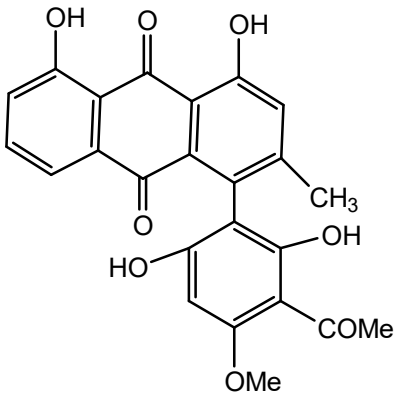
From the pods 10-(physcion-7' –yl) -10 – hydroxy chrysophanol anthrone(**20**), 9-(physcion- 7' –yl) - 5,10– dihydroxy 2-methyl-1-4-anthraquinone(**21**), acacetin 3,5,6,7, 3' 4'-heptahydroxy flavone and kaempferol – 4'–methyl ether along with knipholone(**22**), chrysophanol(**23**), physcion(**24**) and emodin(**10**) were reported.[48] No phytochemical work has been reported on the flowers of this plant.



20. 10-(physcion-7'-yl)-10-hydroxy chrysophanol anthrone



21. 9-(physcion-7'yl)-5,10-dihydroxy-2-methyl-1,4-anthraquinone



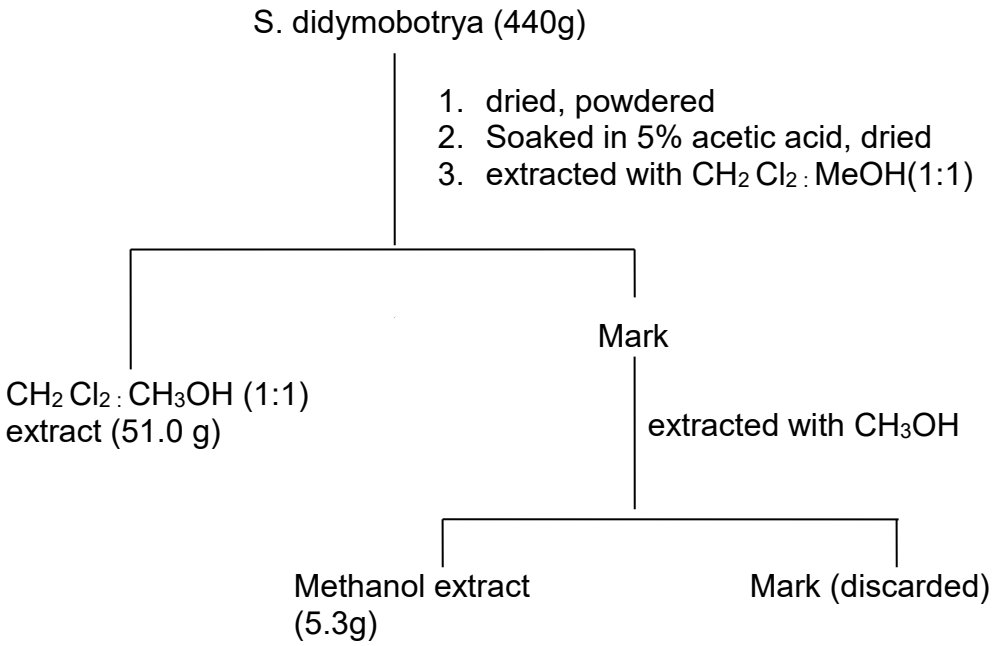
22. Knipholone

5. Objective of the study

The main objective of this research is chromatographic isolation and structural elucidation of secondary metabolites particularly anthraquinones from the flowers of *Senna didymobotrya.*

6. Results and Discussion

The dried and powdered flowers of Senna didymobotrya were extracted with methylene chloride-methanol mixture of 1:1 ratio. This extract when developed on TLC and sprayed with 5% methanolc KOH has shown characteristic changes of colour that indicate the presence of anthraqinones.The brown gummy organic extract was subjected to flash chromatography followed by gel filtration using sephadex LH-20 and preparatory TLC. This study has resulted in the isolation and characterization of 4 anthraquinones, two of which are most likely novel. The known compounds are chrysophanol (**23**) and physcion (**24**)**.** The two novel compounds are 2,2'-chrysophanol-fallacinol (**25**) and 10- (7'- hydroxy- digito-emodin-5'-yl)-10-hydroxy chrysophanol-9-anthrone (**26**). These compounds were identified mainly on the basis of their ^{1}H NMR spectroscopic data in comparison with reported values as well as their similarity on Rf values with authentic samples.



Scheme 2. Method Used to Extract the Plant Material

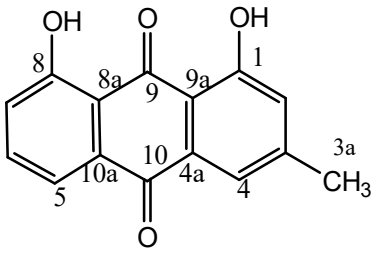
6.1. Compound db-1-6

db-1-6 is a yellow pigment that turned from light yellow into pink colour on TLC when 5% methanolic KOH is sprayed (Rf 0.62, silicagel, pet.ether/EtOAc, 9:1). The ^{1}H NMR spectrum of db-1-6 indicates two chelated hydroxyl resonance at δ 12.08 and 11.95. The aromatic region displayed five aromatic resonance at δ 7.78d (H-5), 7.65t (H-6), 7.60brs (H-4), 7.26d (H-4) and 7.07brs (H-2). More over there is a clear resonance at δ 2.43 for methyl protons.

The ^{13}C NMR showed one methyl carbon at δ 22.6, two oxygenated carbons at 162.8(C-8) and 163.1(C-1), two carbonyl carbons δ at 182.3(C-10) & 192.9(C-9), one methyl substituted carbon δ at 149.7(C-3), five unsubstituted carbons (δ 124.7, C-2; 121.7, C-4; 120.3, C-5; 137.3, C-6 & 124.9, C-7) and four quaternary carbons at δ 134.0, C-4a; 133.7, C-10a, 114.1, C-9a and116.3, C-8a. From these data Compound db-1-6 is a monomer and the proposed structure is shown below.

The proposed structure was supported with COSY, HSQC and HMBC spectra. The COSY correlation indicates the presence of ortho coupling between (H-5 & H-6; H-6 & H-7) and meta coupling between (H-5 & H-7; H-2 & H-4). In addition to this, the COSY spectrum showed correlation between CH_3 &H-2 and CH_3 &H-4.

The proton at δ 7.07(H-2) showed a long range correlation (^3J) with C-9a (δ 114.1), C-4 (121.7), CH_3 (22.6) and ^2J correlation with C-1 (163.1). These and the other HMBC correlation observed for this compound are represented in fig. 1 below.



23. Chrysophanol

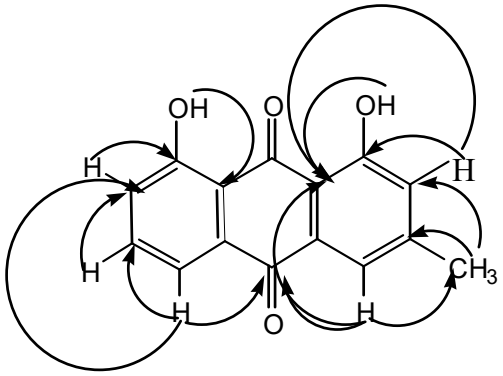


Fig.1 Important HMBC correlations

The HSQC spectrum showed connectivities between the following pairs: δ_H 7.78 and δ_C 120.3 (C-5); δ_H 7.65 and δ_C 137.3 (C-6); δ_H 7.60 and δ_C 121.7 (C-4); δ_H 7.26 and δ_C 124.9 (C-7); δ_H 7.07 and δ_C 124.7 (C-2); δ_H 2.43 and δ_C 22.6 (CH_3). The summary of all observed HSQC and HMBC correlations are compiled in Table 5.

All spectroscopic findings were in good agreement with the suggested structure for db-1-6 to be chrysophanol.

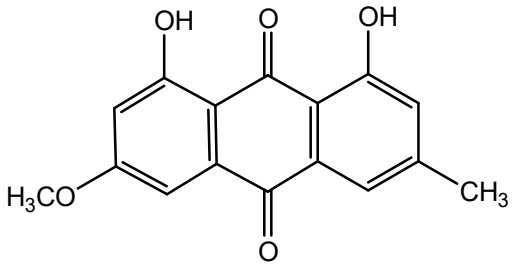
Table 5- Observed correlation in HSQC and in HMBC spectra ($CDCl_3$) of Chrysophanol.

H (δ)	C (δ, J^1)	C (δ, J^n)
HO-1 (12.08)	-	9a (114.1), 2 (124.7), 1 (163.1)
2 (7.07)	2 (124.7)	CH_3-3a (22.6), 1 (163.1), 4 (121.7), 9a (114.1)
CH_3-3a (2.43)	CH_3-3a (22.6)	2 (124.7), 3 (149.7)
4 (7.60)	4 (121.7)	CH3-3a (22.6), 9a (114.1), 2 (124.7), 1 (163.1), 10 (182.3), 9 (192.9)

5 (7.78)	5 (120.3)	6 (137.3), 7 (124.9), 8a (116.3), 9 (192.9), 10 (182.3)
6 (7.65)	6 (137.3)	8 (162.8), 10a (133.7)
7 (7.26)	7 (124.9)	5 (120.3), 8 (162.8), 8a (116.3)
HO-8 (11.95)	-	7 (124.9), 8 (162.8), 8a (116.3)

6.2. Compound db-3-6

Compound db-3-6 was obtained as a yellow solid. It turned in to pink colour on TLC when 5% methanolic KOH is sprayed (R_f 0.59, silicagel, pet.ether/EtOAc, 9:1).The ^{1}H NMR revealed the presence of two chelated hydroxyl resonance at δ12.19 and 11.98, four aromatic protons at δ 7.64s for H-4, 7.32d for H-5, 7.07s for H-2 and 6.67s for H-7. In addition to these signals there is a clear resonance at δ 3.92s and 2.40s for OCH_3 and CH_3 respectively. The structure of this compound was deduced to be physcion in comparison with reported values[8] as well as co-TLC with authentic sample.



24. Physcion

6.3. Compound db-7-7

This is an orange –yellow compound (m.p. above 300^0c) turned to violet on a TLC plate upon spraying with 5% KOH in methanolic solution (Rf 0.42, CHCl3/MeOH, 9:1). The UV spectrum showed absorption maxima at 270,290 and 440nm, suggesting a quinoid chromophore. The IR spectrum showed bands for a hydroxyl, free and chelated carbonyl groups at 3620, 1710 and 1602cm^{-1} respectively. The ^{1}H NMR spectral data indicated the presence of four chelated hydroxyl groups (12.24s, 12.18s, 12.09s and 12.08s), seven aromatic resonance at δ 6.70d, 7.32d, 7.38d, 7.70t, 7.78s, 7.81s &7.85d. The signal at δ 4.82d, 3.95s and 2.62s correspond to CH_2OH, OCH_3 and CH_3 respectively. All these data can't be accommodated by the monomer, therefore the compound should be a dimmer, as a first proposal.
In order to know the plausible structure of the compound, consultation was made to the literature value of chrysophanol, physcion, aloe-emodin and fallacinol which is given in table-6-below.

Table.6. ^{1}H NMR (400 MHz, $CDCl_3$) Spectral data of chrysophanol (**23**), physcion (**24**), aloe-emodin & fallacinol

H	δ (ppm) chrysophanol	δ (ppm) physcion	δ (ppm) aloe-emodin	δ (ppm) fallacinol
2	7.07brs	7.04s	7.36brs	7.30brs
$3CH_3$	2.43s	2.45s	_	_
$3CH_2OH$	_	_	4.80s	4.80s
4	7.60s	7.57brs	7.79brs	7.75brs
5	7.78d	7.32d	7.82dd	7.38d
6	7.65t	_	7.68t	_
$6OCH_3$	_	3.92s	_	3.90s
7	7.26d	6.60d	7.30dd	6.70d

On the basis of the obtained spectral data and the literature value of Table 6, two possible assignments can be made as it is compiled in Table-7 below.

Table 7. ^{1}H NMR (400 MHz, $CDCl_3$) Spectral data of compound 25A and 25B (db-7-7).

H	δ (ppm) of 25A	δ (ppm) of 25B
1-OH*	12.28s	12.28s
3-CH_3	2.62s	2.62s
4	7.81s	7.78s
5	7.38d	7.85s
6- (OCH_3),6	3.95s	7.70t
7	6.70d	7.32d
8-OH*	12.18s	12.18s
1'-OH*	12.09s	12.09s
3'-CH_2OH	4.82d	4.82d
4'	7.78s	7.81s
5'	7.85d	7.38d
6', (OCH_3)	7.70t	3.95s
7'	7.32d	6.70d
8'-OH	12.08s	12.08s

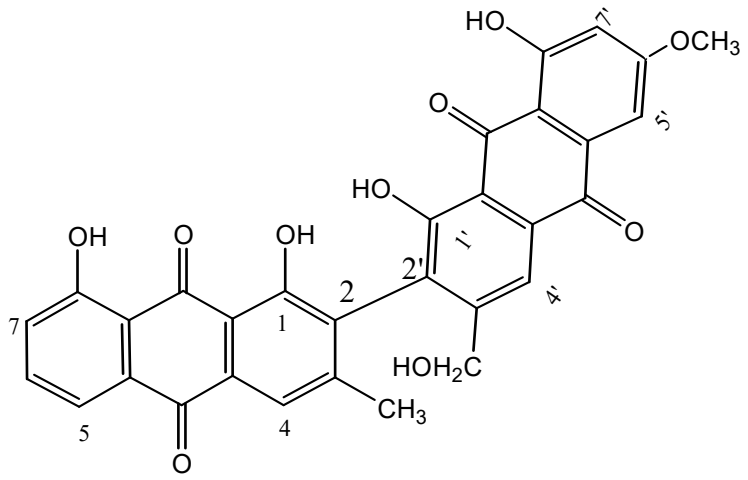
* Signals may interchange

When we compare the chemical shift value of the moieties of compound db-7-7 (i.e. for first column) with that of physcion and aloe-emodin of Table-6, one of the moieties δ at 7.32d (H-7'), 7.70t (H-6'), 7.85d (H-5'), &7.78s (H-4') showed greater resemblance with the chemical shift value of aloe-emodin (δ at 7.30d (H-7), 7.68t (H-6), 7.82d (H-5) & 7.78s (H-4).

H	δ(ppm) of aloe- emodin moieties	δ(ppm) of aloe-emodin
4'	7.78s	7.78s
5'	7.85d	7.82d
6'	7.70t	7.68t
7'	7.32d	7.30d

But the other moiety δ at 7.81s (H-4) & 6.70d (H-7) could not show the expected resemblance with that of physcion δ at 7.57s (H-4), & 6.60d (H-7).

H	δ(ppm) of physcion moieties	δ(ppm) of physcion
4	7.81s	7.57s
5	7.38d	7.32d
7	6.70d	6.60d



25A. 2,2'-physcion-aloe-emodin

When we consider the second alternative, there is a relatively concordant chemical shift values between the moieties of db-7-7 (column-2) and literature value of the monomers (i.e. chrysophanol & fallacinol).

H	δ(ppm) of chrysophanol moieties	δ(ppm) of chrysophanol
4	7.78s	7.60s
5	7.85d	7.78d
6	7.70t	7.65t
7	7.32d	7.26d

The signals at 7.78s, 7.85s, 7.70t and 7.32d are attributed to 4-H, 5-H, 6-H & 7-H of a chrysophanol moiety which is relatively agreed with the chemical shift value of chrysophanol δ at 7.60s (4-H), 7.78d (5-H), 7.65t (6-H) and 7.26d (7-H).

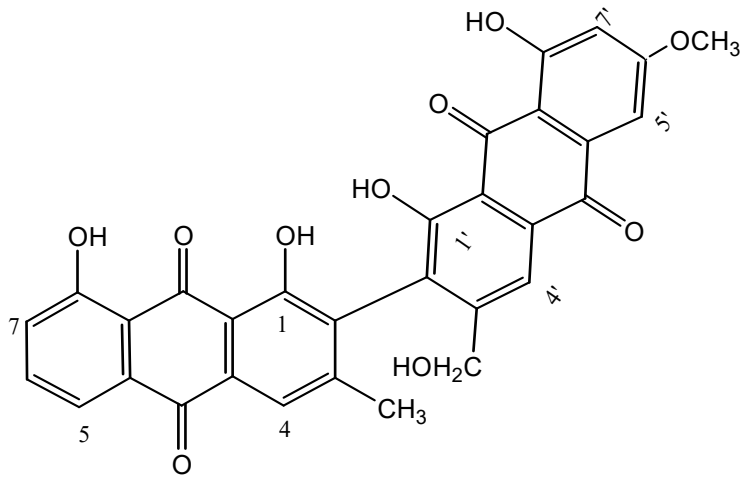
H	δ(ppm) of fallacinol moieties	δ(ppm) of fallacinol
4’	7.81s	7.75s
5’	7.38d	7.38d

7’	6.70d	6.70d

The two meta coupled protons (6.70d for 7’-H and 7.38d for 5’-H) together with 7.81s for 4’-H are assignable to the protons of a fallacinol moiety which is also showing greater resemblance with the chemical shift value of fallacinol δ at 6.70d (7-H), 7.38d (5-H) and 7.75 (4-H).

The down field signals for H-4 (7.78s), H-5 (7.85d), H-4’ (7.81s) and H-5’ (7.38d) is another supportive information that indicates the non-oxanthrone nature of both the chrysophanol and fallacinol moiety. More over the signals for 2-H on chrysophanol and 2’-H on fallacinol have disappeared in the spectrum of db-7-7. Therefore the two monomeric units should be linked at C-2 of chrysophanol and C-2’ of the fallacinol.

Based on these evidences the preferred structure of the compound was deduced to be 2-2’-chrysophanol-fallacinol (**25B**).



25B. 2,2'-chrysophanol-fallacinol

6.4 Compound db-4-7

This is a yellow pigment that turned red on a TLC plate upon spraying with 5% KOH in methanolic solution. Compound db-4-7 (m.p. above 300^0c) displayed UV absorption maxima at λ 440,390 and 275nm, suggesting the presence of a quinoid

chromophore. The IR spectrum showed absorptions at 3620, 1710, 1601cm^{-1} indicating the presence of hydroxyl, non-chelated and chelated carbonyl groups respectively. The ^{1}H NMR spectrum showed the presence of three chelated hydroxyl groups (δ 12.47, 12.38, 11.86), two aromatic methyl signals at δ(2.47, 2.28) and 8 aromatic protons at δ 7.64br s, 7.42t, 7.32 br s, 7.13br s, 6.98d, 6.92d, 6.79br s, and 6.76brs which can be accommodated on a bianthraquinone skeleton.

Table. 8 : Comparison of ^{1}H NMR (400 MHz, $CDCl_3$) anthrone moietyof db-4-7 & compound 19

H	δ (ppm) db-4-7	δ(ppm) compound 19[66]
1-OH	12.38s	12.30s
2	6.79s	7.01brs
3-CH_3	2.47s	2.30s
4	6.76s	6.80brs
5	6.92d	6.95d
6	7.42t	7.55t
7	6.98d	7.12d
8-OH	12.47s	12.38s
1'-OH	11.86s	12.32s
2'	7.13s	7.21s
3'-CH_3	2.28s	2.50s

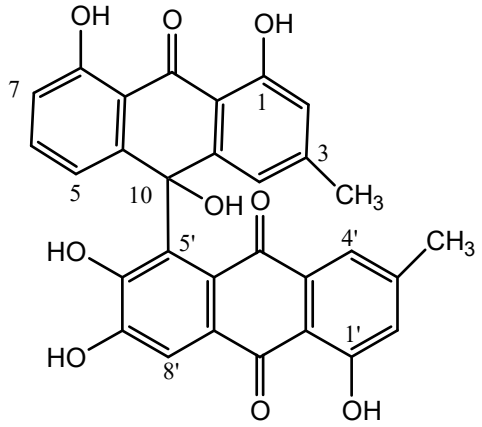
4'	7.64s	7.64s
5’	-	7.40
6’	-	3.80
8'-H(OH)	7.32s	12.40

The signals at 7.42t, 6.98d, 6.92d 6.79s and 6.76s are assignable to 6-H, 7-H, 5-H, 2-H and 4-H of a chrysophanol moiety. These resonances have strong resemblance with the chrysophanol anthrone of compound 19.[66]

7.64s for 4’-H, 7.32s for 8’-H and 7.13s for 2’-H are assignable to the protons of the other moiety.

The relatively upfield signals for H-4 (6.76s) and H-5 (6.92d) indicate the oxanthrone nature of the chrysophanol moiety. This explains that the other moiety is attached on C-10 of the chrysophanol skeleton. The signal for 5’ on the digito-emodin moiety had disappeared in the spectrum of db-4-7 which indicated that, the chrysophanol moiety has coupled on C-5’ position of the other moiety. Therefore the two monomeric units are linked at C-10 of chrysophanol and C-5’ of the digito-emodin moiety.

The structure of the compound was proposed to be



26. 10-(7-hydroxy-digito-emodin-5’-yl)-10-hydroxy chrysophanol-9- anthrone

7. Experimental

7.1. General

The flower of S. didymobotrya was collected near Assebe Teferi on October 14, 2004. The specimen of the plant is deposited at the National Herbarium of A.A.U. with a voucher No. AH.2&3.

- Analytical TLC was done with silicagel 60 F_{254} (merck) coated on aluminium sheets, 0.20nm thickness.
- Preparative TLC was performed using silicagel 60 PF $_{254+366}$ (merck), 1 mm thickness.
- Flash, silicagel column chromatography was done using silicagel 60, particle size 0.063-0.200
- Sephadex (LH-20, $CHCl_3$ – MeOH 2:1).
- UV spectra were performed by GENEY'S 2 PC spectrometer (200-800 nm) at room temperature.

- IR spectra were measured on a Perkin-Elmer Bx spectrometer 400-4000 cm^{-1} in KBr pellets.
- Melting points were determined on electrothermal digital m.p. apparatus.
- NMR spectra in $CDCl_3$ (400 MHz) were run on a Bruker Avance Spectrometer.

7.2. Extraction and Isolation

The dried and ground plant material (440 g) was soaked in 5% acetic acid for 24 hrs, and allowed to dry. It was extracted with a 1:1 ratio of CH_2Cl_2 : MeOH and the extract was freed of solvent to give 51 g dark gummy residue which on TLC (Solvent system $CHCl_3$) showed 4 coloured spots.

This extract was adsorbed with 40 g silicagel and after being dried applied to a column, packed with 200 g silicagel (impregnated with 5% oxalic acid). The column was eluted with only chloroform to collect 13 fractions (each 250 ml). All the 13 fractions showed characteristic colored spots on TLC upon spraying with 5% methanolic KOH. The first and the second fractions were labeled as Fr-I and Fr-II respectively. Fractions 3-8 were combined based on their TLC similarity, and were labeled as Fr-III. Fractions 9-13 were also combined and labeled as Fr-IV.

Fraction I was applied to a medium column of silicagel (PE/EA,9:1) to collect 13 fractions(each 50ml).

On the basis of TLC similarity, fractions 1-3 were discarded, fractions 4-6 combined to give 1.1 and fractions 7-13 were combined to give 1.2.

1.1 was subjected to column chromatography in which 30g of silicagel was packed with PE/EA, 9:1 and gave six fractions(each 15ml).

From these fractions the last three were combined and applied to sephadex (CH_2Cl_2:MeOH, 2:1) to collect 8 fractions. Fractions 4-8 combined to yield compound db-1-6.

1.2 was applied to column chromatography packed with 40 g silica gel (PE/EA, 9:1) solvent system and gave 20 fractions(each 50 ml). Frs 5-16 were combined and subjected to sephadex (CH_2Cl_2 :MeOH, 2:1) to collect 17 fractions(each

15ml). Fractions 9-17 were combined and subjected to PTLC, PE/EA(9:1) to collect two fractions from the two bands. They were labeled as compound db-2-6(the upper band) and compound db-3-6 (the lower band).

Fr-II was also applied on a column packed with chloroform. The solvent system used to elute the column was chloroform for frs1-35 and chloroform-ethyl acetate(1:1) for frs 36-42.

Fractions 15-31 were combined to give 2.1 and fractions 36-42 were combined to give 2.2.

Fraction 2.1 was loaded on Sephadex LH-20 column and eluted with CH_2Cl_2-MeOH (2:1) to yield 12 fractions. Fractions 6 -12 were combined and subjected to column chromatography PE/EA, 9:1 to collect 5 fractions(15ml). From these five fractions the last three were combined and yield compound db-5-6.

Fraction 2.2 was subjected to separation on sephadex and then chromatographed on PTLC, solvent PE/EA(1:1) to give compound db-4-7.

Fr-III showed a dark brown colour upon spraying with 5% methanolic KOH and is kept for further investigation.

Fraction IV were subjected to chromatography over sephadex LH-20 (2:1 CH_2Cl_2 /MeOH) to collect 12 fractions.

Compound db-6-7 and db-7-7 were isolated from these 12 fractions in the following manner. Fractions 5-12 were combined and chromatographed on PTLC, $CHCl_3$/ MeOH(9:1) so that clean separation of compound db-6-7 and db-7-7 was possible. As a remark, from these 6 compounds db-1-6, db-2-6 and db-5-6 showed the same spectroscopic data and therefore taken as one compound.

8.0 Spectral data

Chrysophanol (**23**)

Yellow solid melting point 195-196 ^{0}c, IR V_{max} (KBr) cm^{-1}, 3417, 2366, 1628, 1459, 1373, 1274, 1206,1157, 754, 612. ^{1}H NMR (400 MHz, $CDCI_3$): δ12.08 (IH,s, OH-1), 11.95 (IH, s, OH-8), 7.78 (IH, d, H-5), 7.65 (IH, t, H-6), 7.60 (IH, s,H-4), 7.26 (IH, d, H-7), 7.07 (IH, brs,

H-2), 2.43 (3H, s,CH_3), ^{13}C NMR ($CDCl_3$): δ 22.6 (CH_3), 114.1 (C-9a), 116.3 (C-8a), 120.3 (C-5), 121.7 (C-4), 124.7 (C-2), 124.9 (C-7), 133.7 (C-10a), 134.0 (C-4a), 137.3 (C-6), 149.7 (C-3), 162.8 (C-8), 163.1 (C-1), 182.3 (C-10), 192.9 (C-9).

Physcion (**24**)

Yellow solid melting point 192 ^{0}c, IR V_{max}(KBr) cm^{-1}, 3416, 2910, 2366, 1623, 1474, 1373, 1272, 1217, 1159, 1034, 769, 617, 471. ^1H NMR (400 MHZ, $CDCl_3$), δ 12.32 (IH, s, OH-1), 12.10 (IH, s, OH-8), 7.64 (IH, s, H-4), 7.32 (IH, d, H-5), 7.07 (IH, s, H-2), 6.67 (IH, s H-7), 3.92 (3H, s, OCH_3), 2.40(3H, s, CH_3). ^{13}C NMR ($CDCl_3$): δ22.4 (CH_3), 56.5 (OCH_3), 114.1 (C-9a), 110 (C-8a), 107.2 (C-5), 108.6 (C-4), 124.9 (C-2), 121.9 (C-7), 133.6 (C-10a), 135.7 (C-4a), 162.9 (C-6), 148.8 (C-3), 167 (C-1), 166 (C-8), 182.4 (C-10), 191.2 (C-9).

2-2' - Chrysophanol – Fallacinol (**25B**)

^1H NMR (400MHZ, $CDCl_3$): δ 12.28 (1H, s, OH-1), 12.18s (1H,5-OH-8), 12.09 (1H, s -OH-1'), 12.08 (1H, s, OH-8'), 7.81 (1H, s, 4'H) 7.40 (1H, s,5'H), 6.70 (1H, d, 7'H), 7.79 (1H, s, 4H), 7.88 (1H, d, 5H), 7.70 (1H, t, 6H), 7.32 (1H, d, 7H), 4.82 (2H,d, 3'CH_2OH), 3.94 (3H, s, 6'OCH_3), 2.62 (3H, s, 3CH_3).

Compound db-4-7 (**26**)

^1H NMR (400 MHz, $CDCl_3$): δ 12.47 (1H, s, OH-1), 12.38 (1H, s, OH-8'), 11.86 (1H, s, OH-1'), 6.79 (1H,s, 2H), 6.76 (1H, S, 4H), 6.92 (1H, d, 5H), 7.42 (1H, t, 6H), 6.98 (1H, d, 7H), 7.13 (1H, s, 2'H), 7.64 (1H, s, 4'H), 7.32 (1H, s, 8'H), 2.47 (3H, s, 3CH_3), 2.28 (3H,s, 3'CH_3).

9. Conclusions and Recommendation

From the flowers of *Senna* didymobotrya, four compounds have been isolated and characterized, two of which are likely to be novel. The likely novel compounds are 2,2'-chrysophanol-fallacinol and 10-(7-hydroxy-digitoemodin-5'-yl)-10-hydroxy chrysophanol-9-anthrone.The known compounds are common anthraquinones chrysophanol & physcion. The known compounds were identified by co-chromatography with authentic samples and comparing their ^{1}H NMR spectrum with that reported in the literature. The likely new compounds were obtained in low yield and this limited our efforts to do more experiments. The study yielded a number of un identified pigments whose structures should be elucidated in the future.

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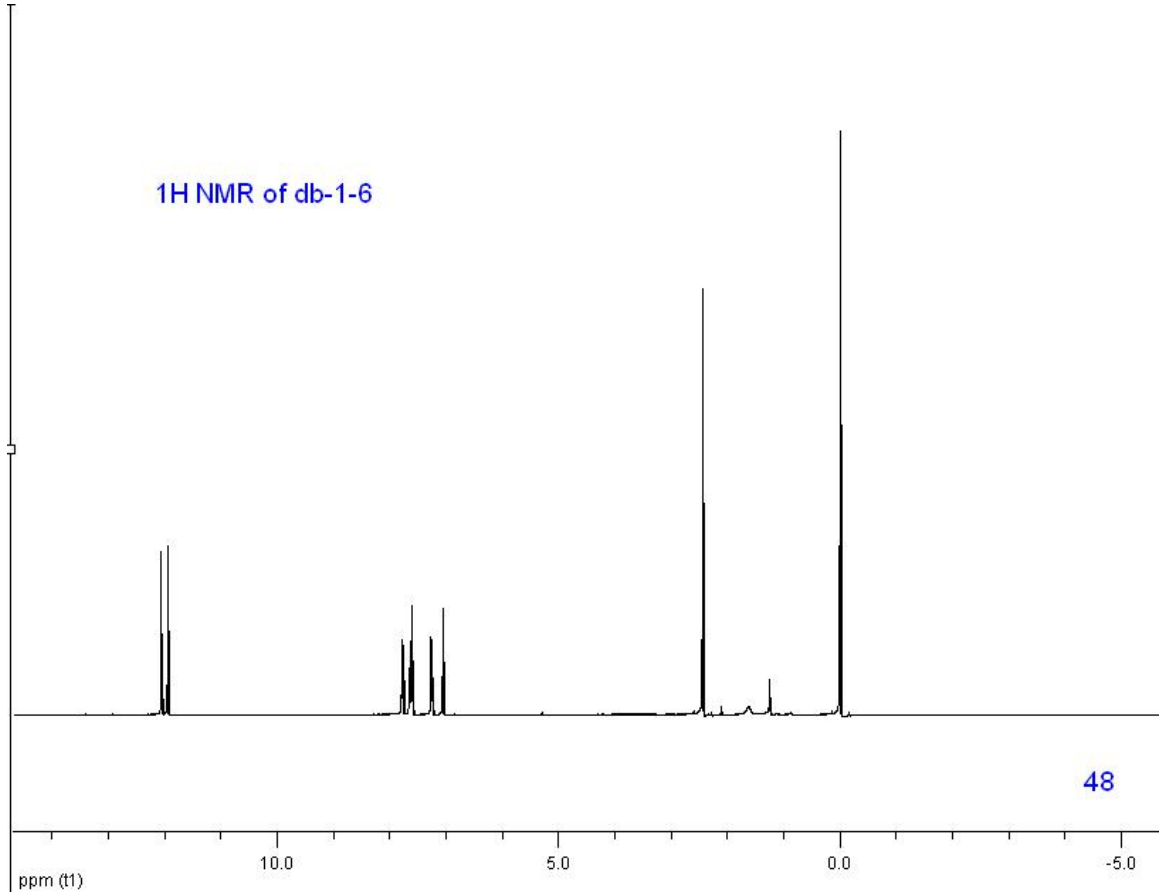
Abstract

Phytochemical investigation on the flowers of *Senna didymobotrya* led to the isolation and characterization of the known anthraquinones chrysophanol (**23**) and physcion (**24**); two most likely new anthraquinones 2,2'-chrysophanol-fallacinol (**25B**) and 10-(7-hydroxy-digito emodin-5'-yl)-10-hydroxy chrysophanol-9-anthrone (**26**).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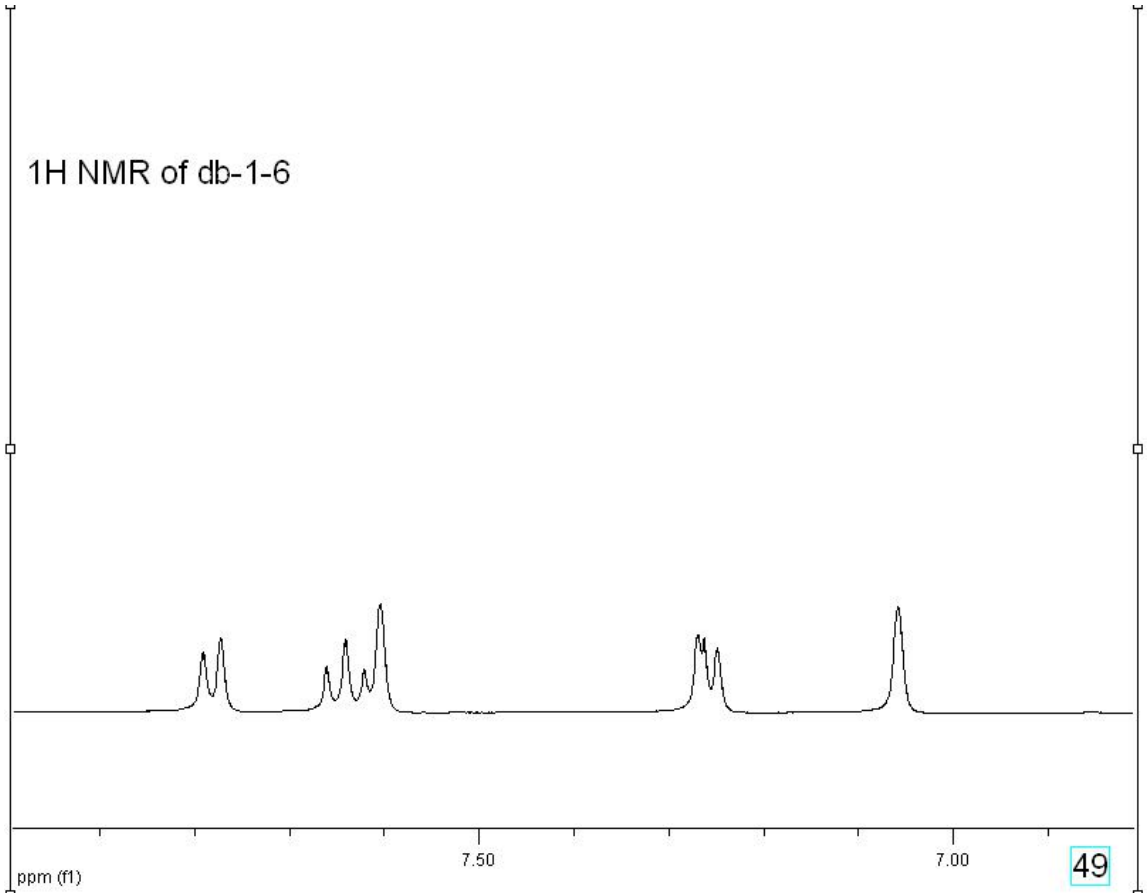
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ppm (f1)
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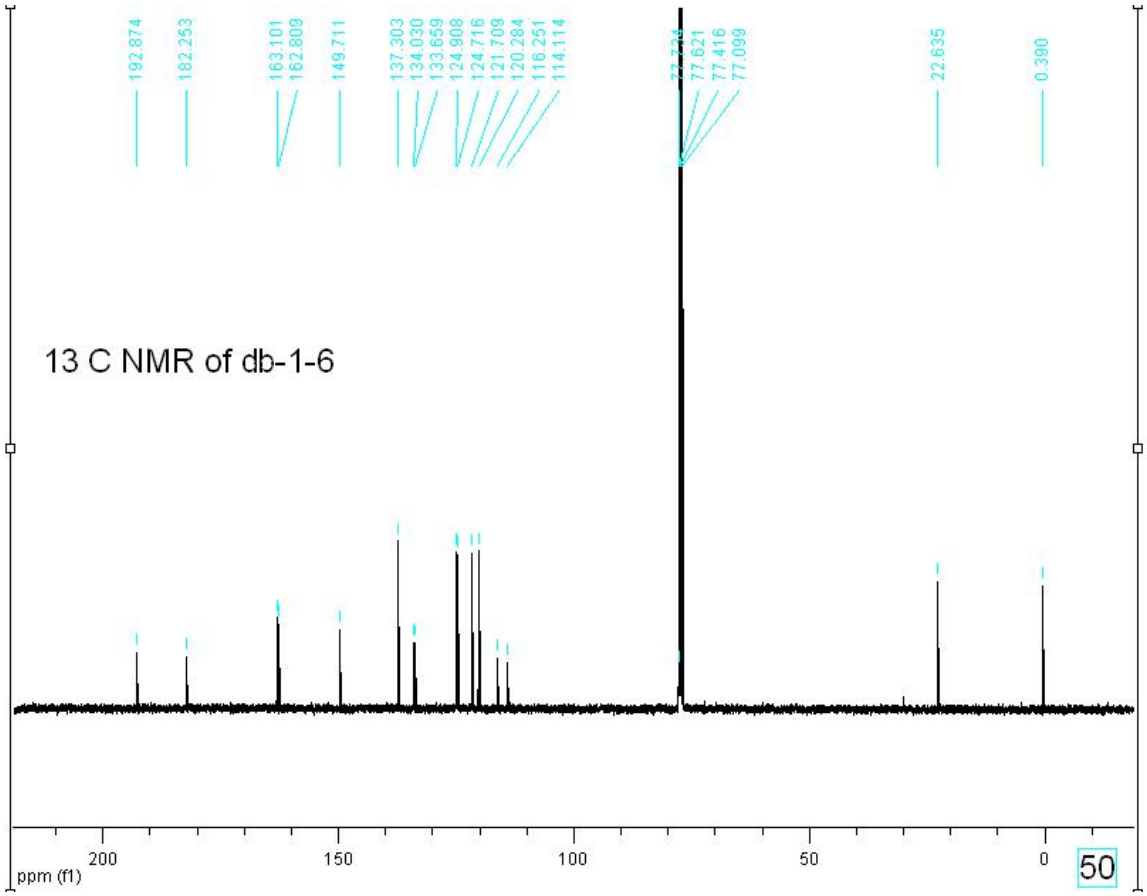
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116.251
114.114
77.621
77.416
77.099
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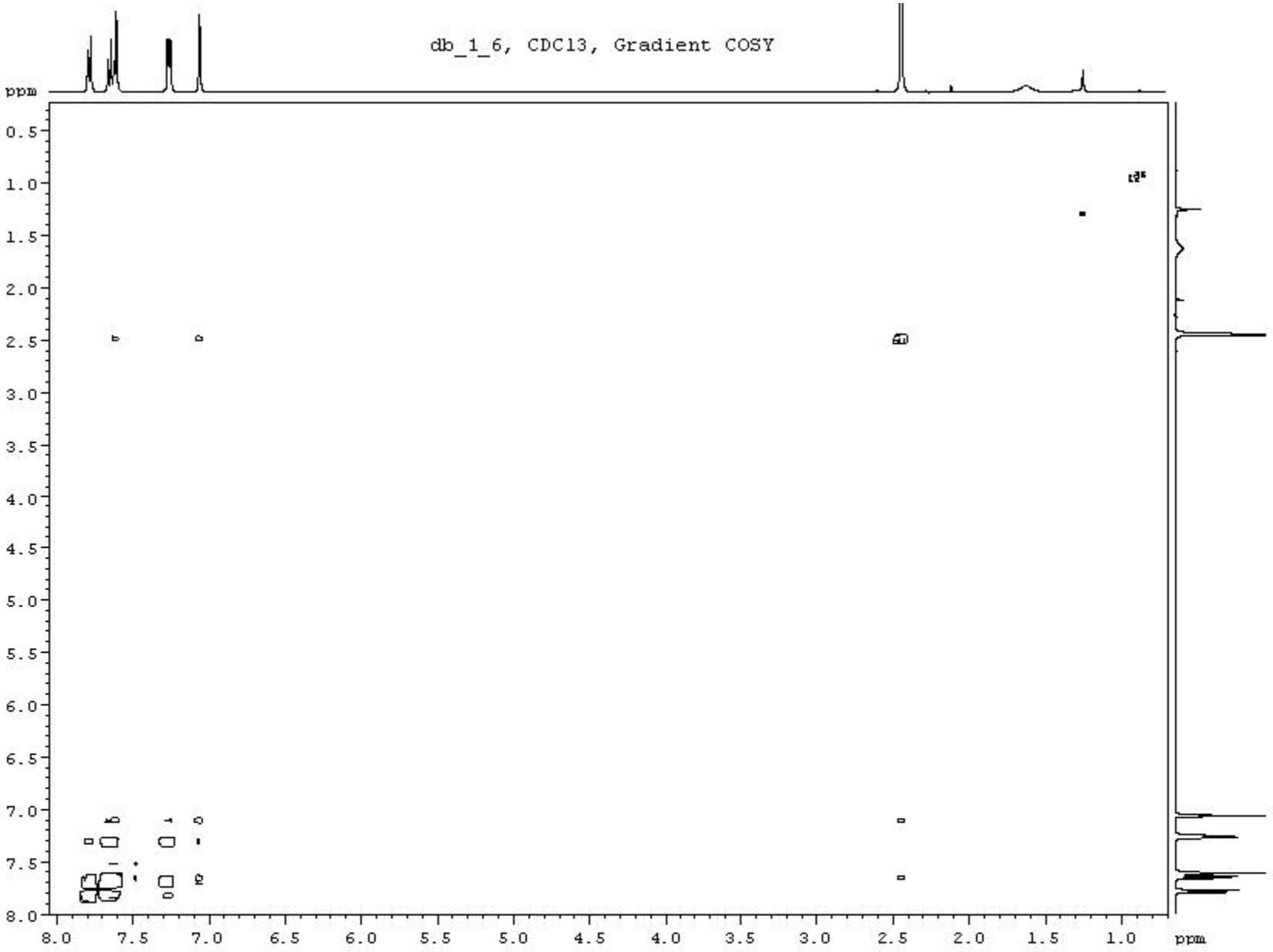
100
50
0
ppm (f1)
50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4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.0
7.5
7.0
6.5
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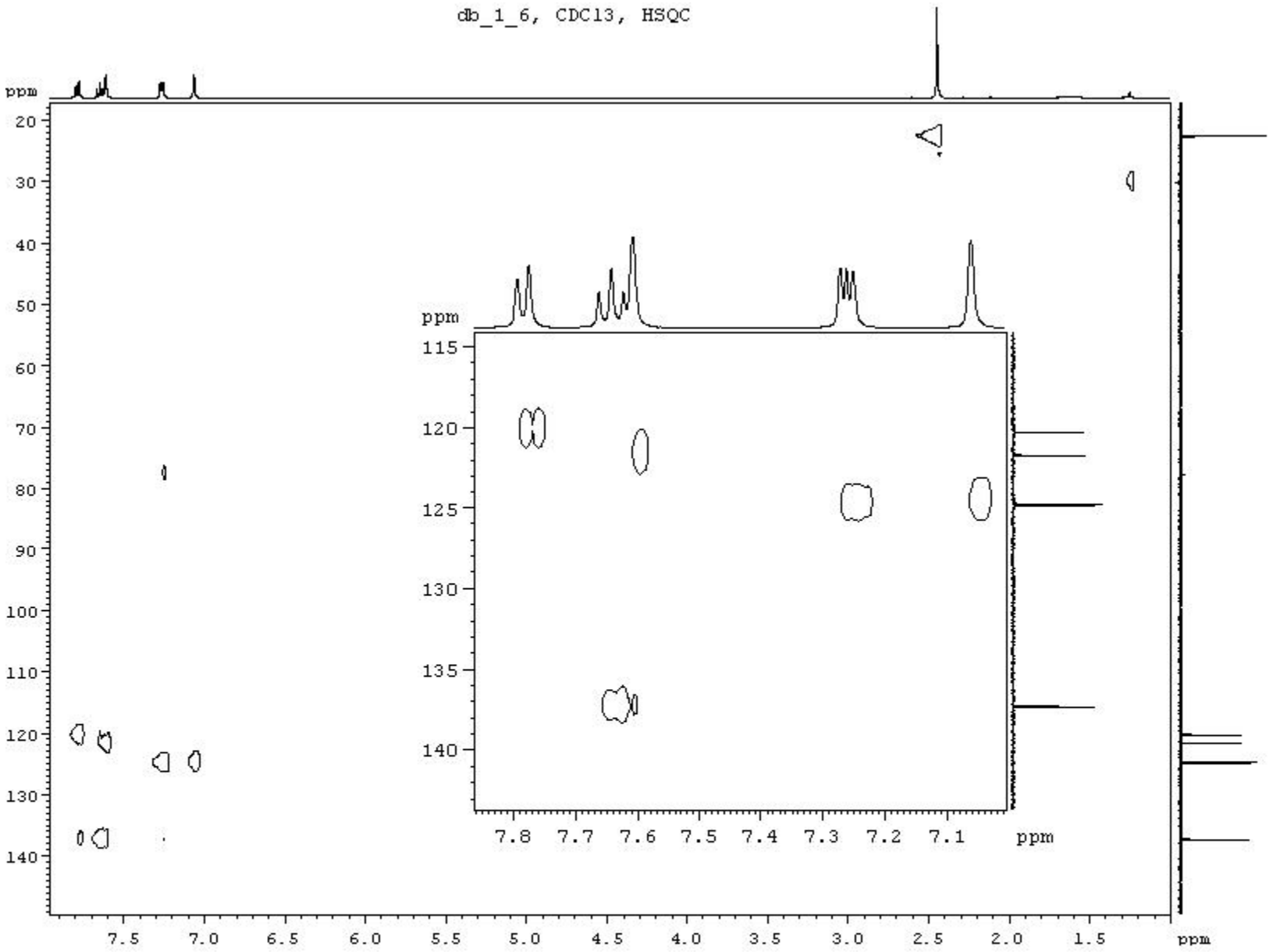
1.0
ppm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50
60
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90
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110
120
130
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7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
ppm
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120
125
130
135
140
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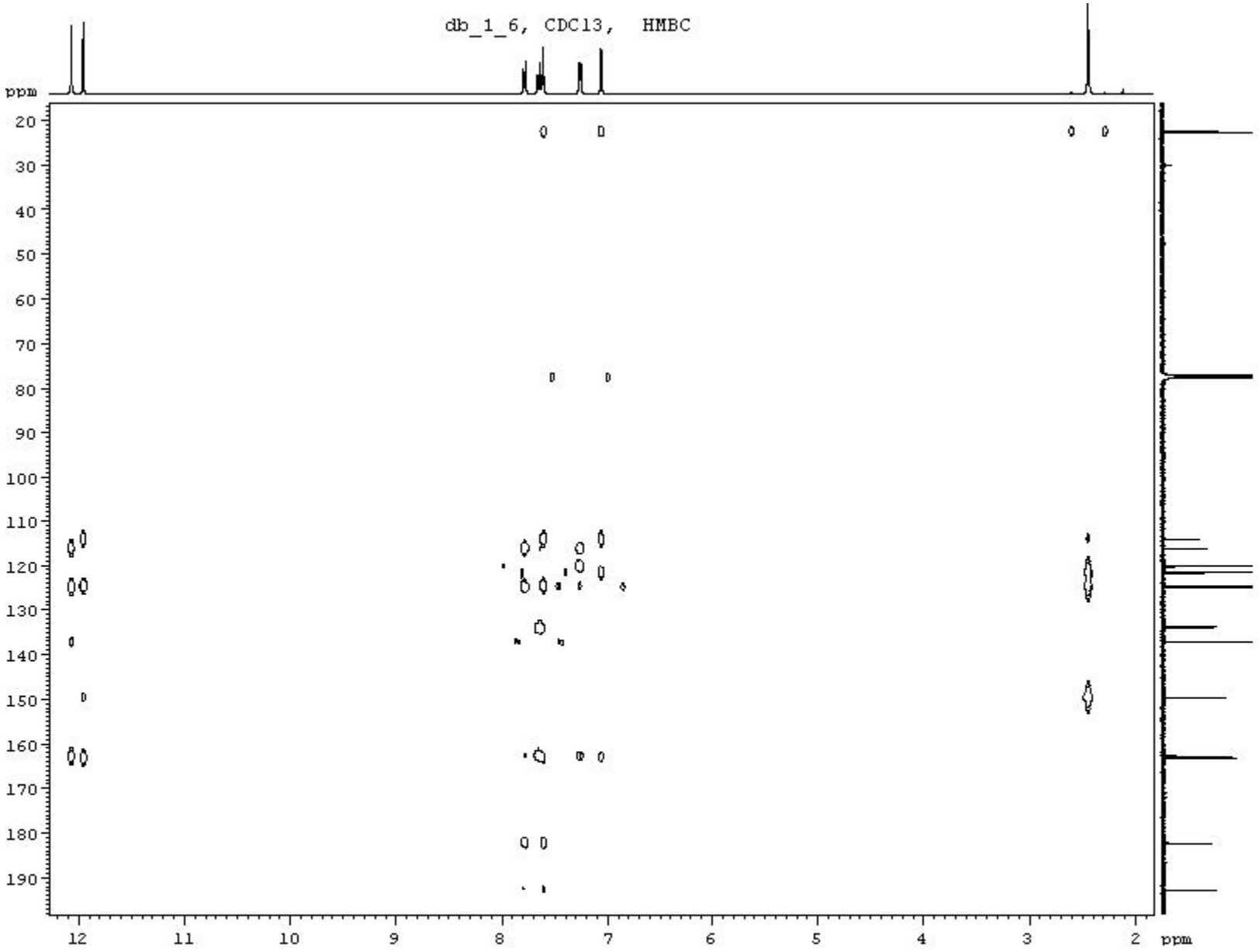
7.6
7.5
7.4
7.3
7.2
7.1
ppm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50
60
70
80
90
100
110
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160
170
180
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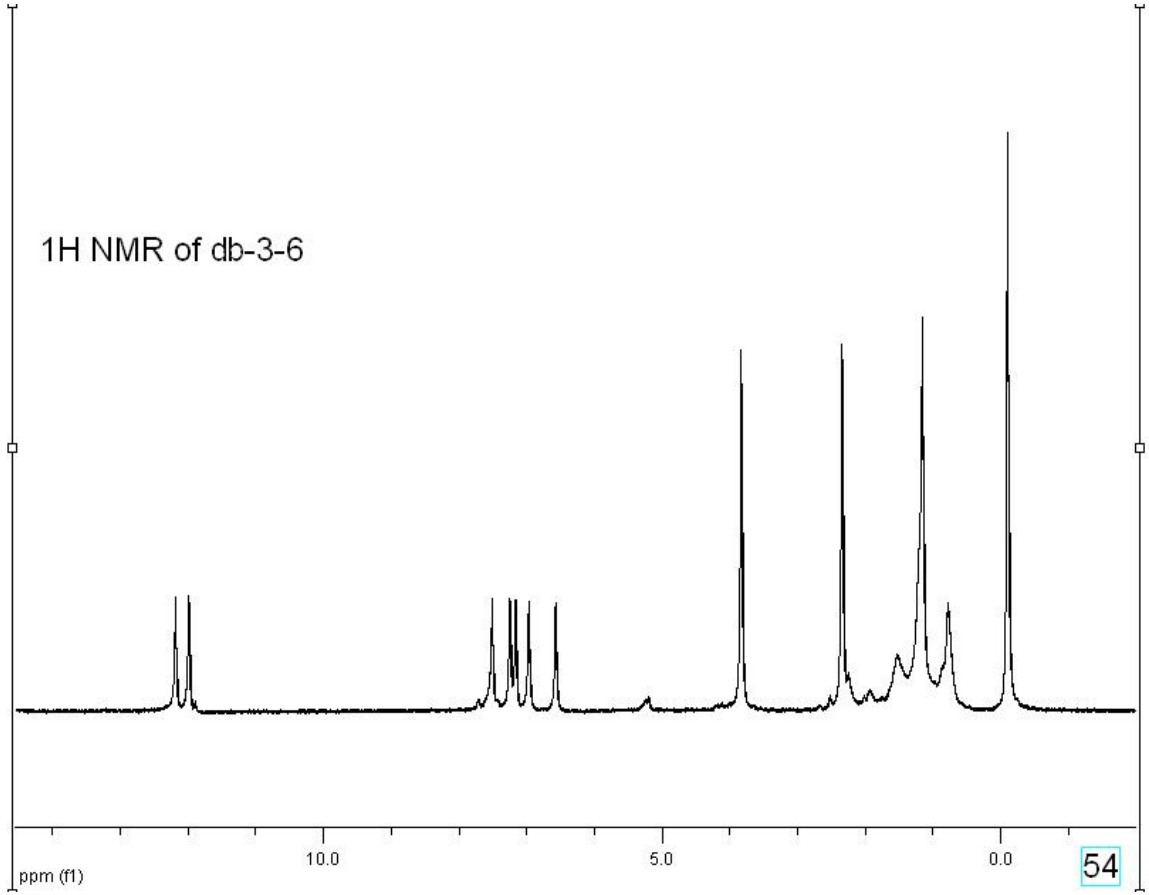
11
10
9
8
7
6
5
4
3
2
ppm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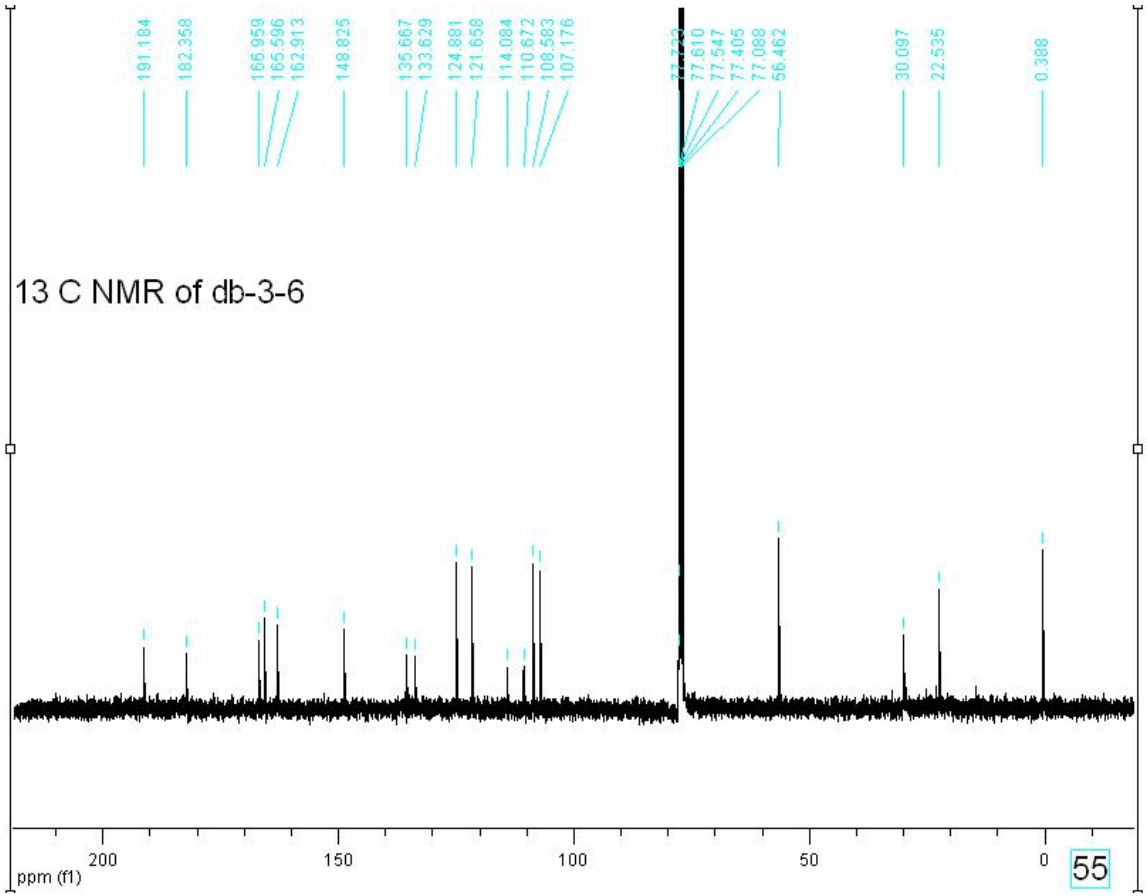
0.0
ppm (f1)
54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162.913
148.825
135.667
133.629
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114.084
110.672
108.583
107.176
77.722
77.610
77.547
77.405
77.088
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30.097
22.535
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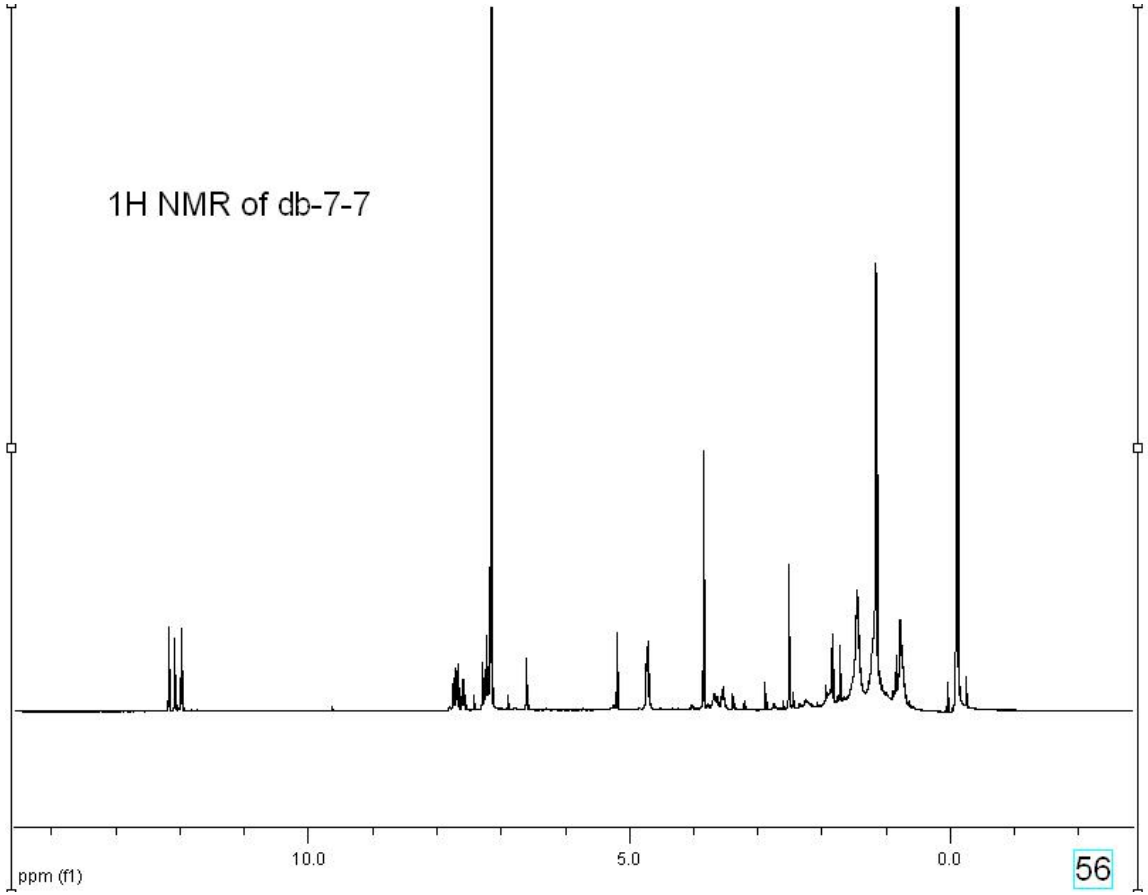
100
50
0
ppm (f1)
55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5.0
0.0
ppm (f1)
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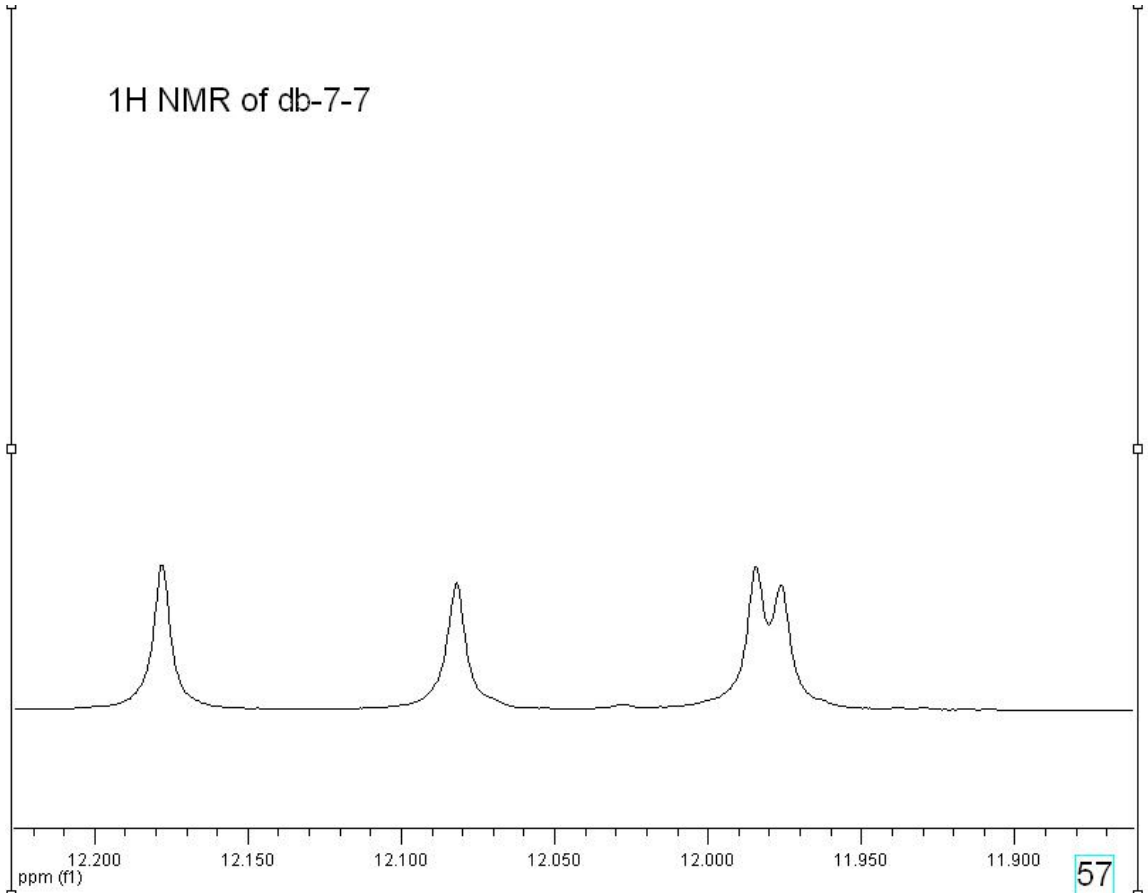
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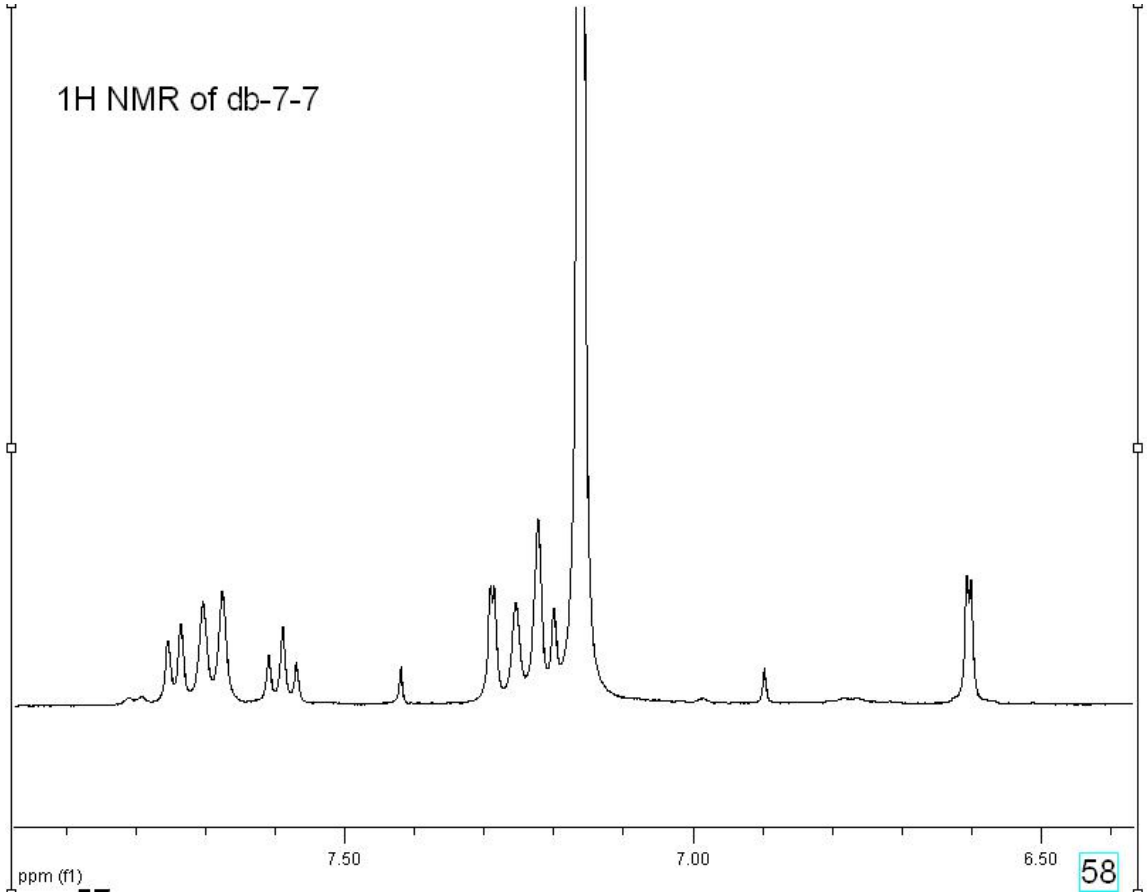
12.050
12.000
11.950
11.900
ppm (f1)
57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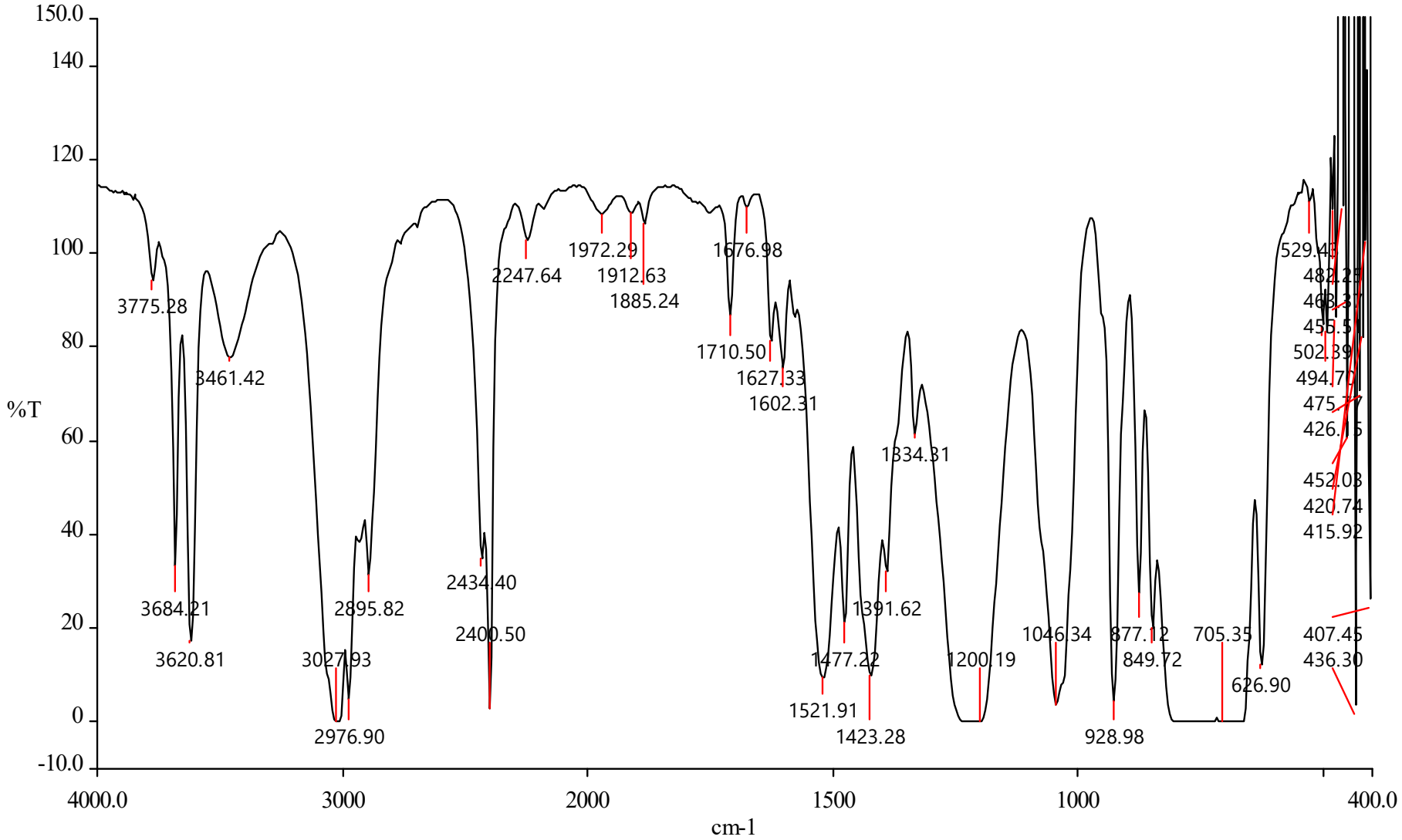
6.50
ppm (f1)
58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%T
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3000
2000
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1000
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cm-1
3775.28
3461.42
3684.21
3620.81
3027.93
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2400.50
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1972.29
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1710.50
1627.33
1602.31
1521.91
1477.22
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1334.31
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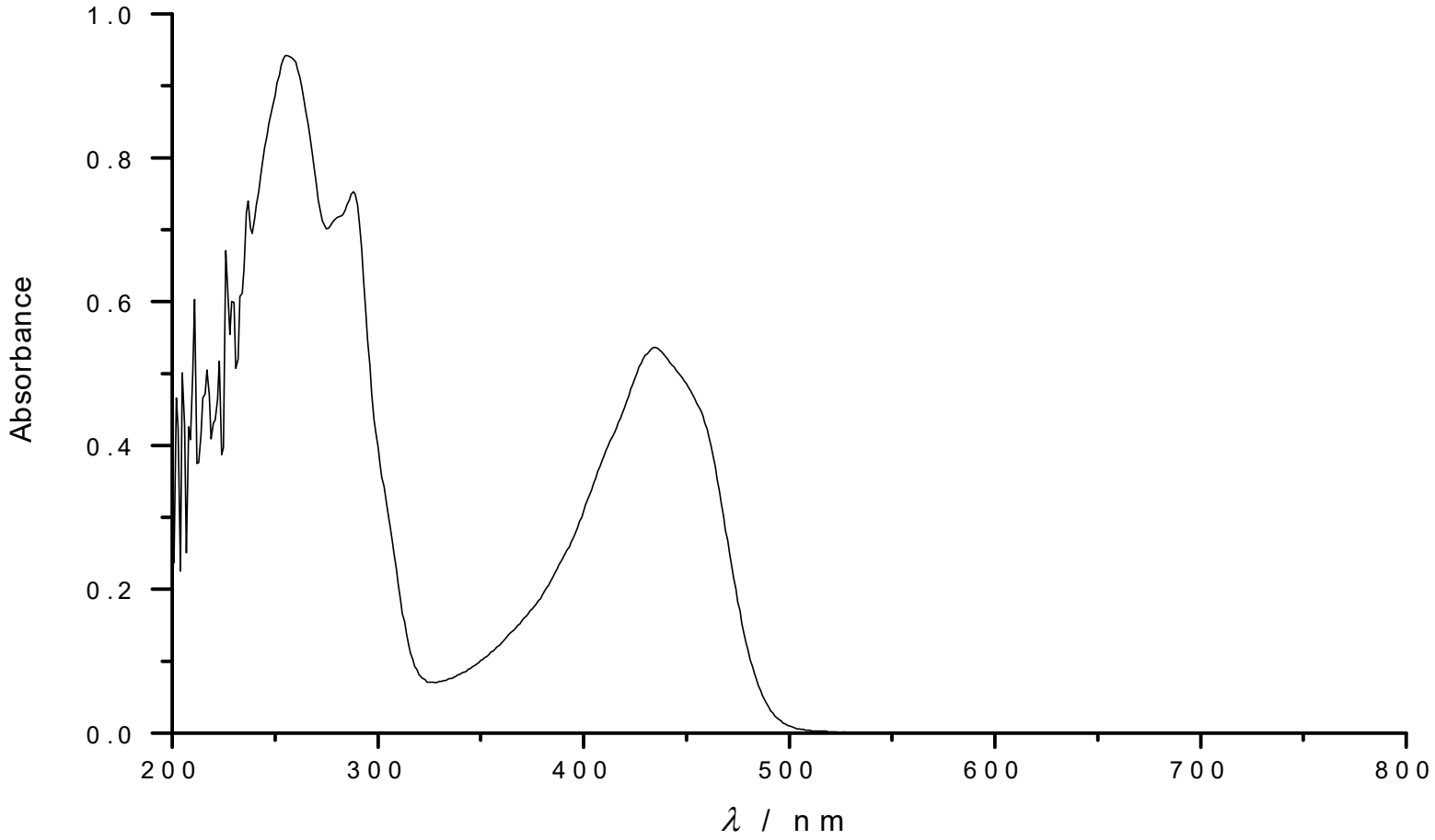
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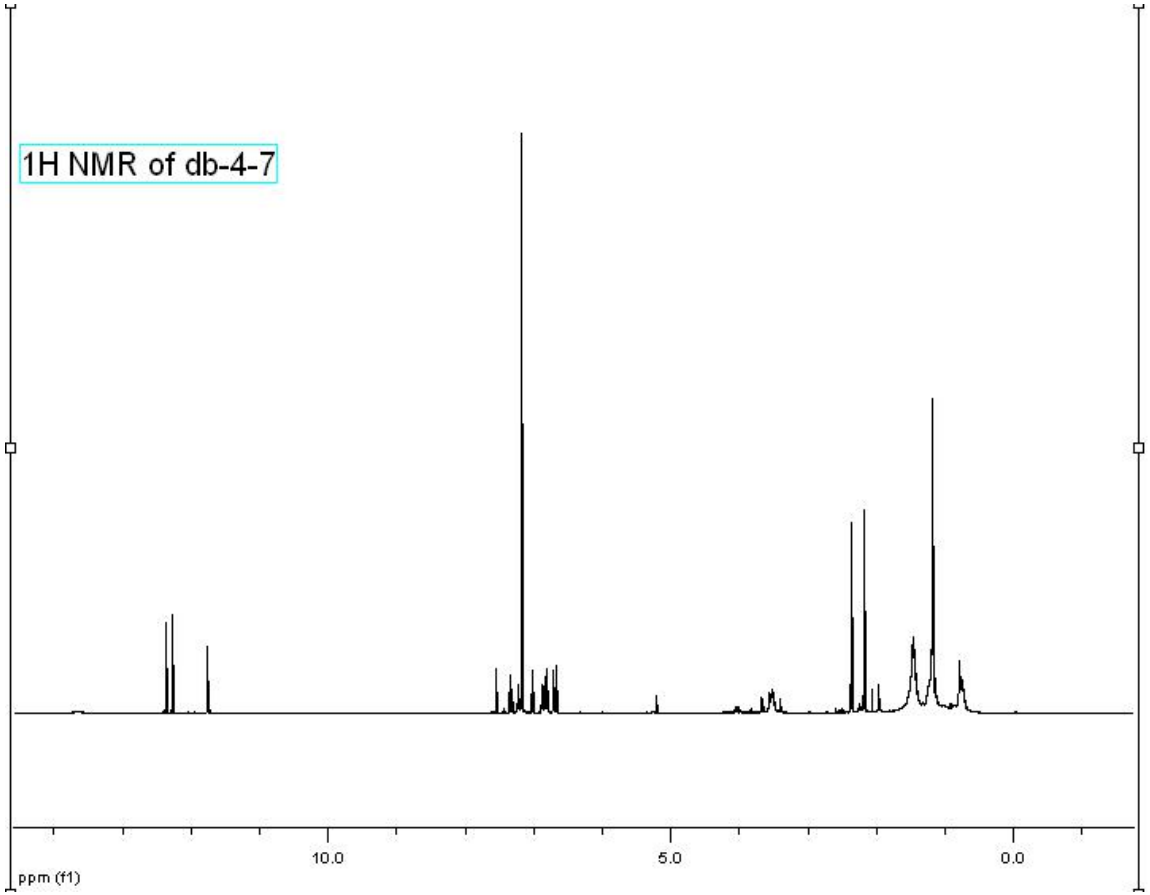
200
300
400
500
600
700
800
λ / nm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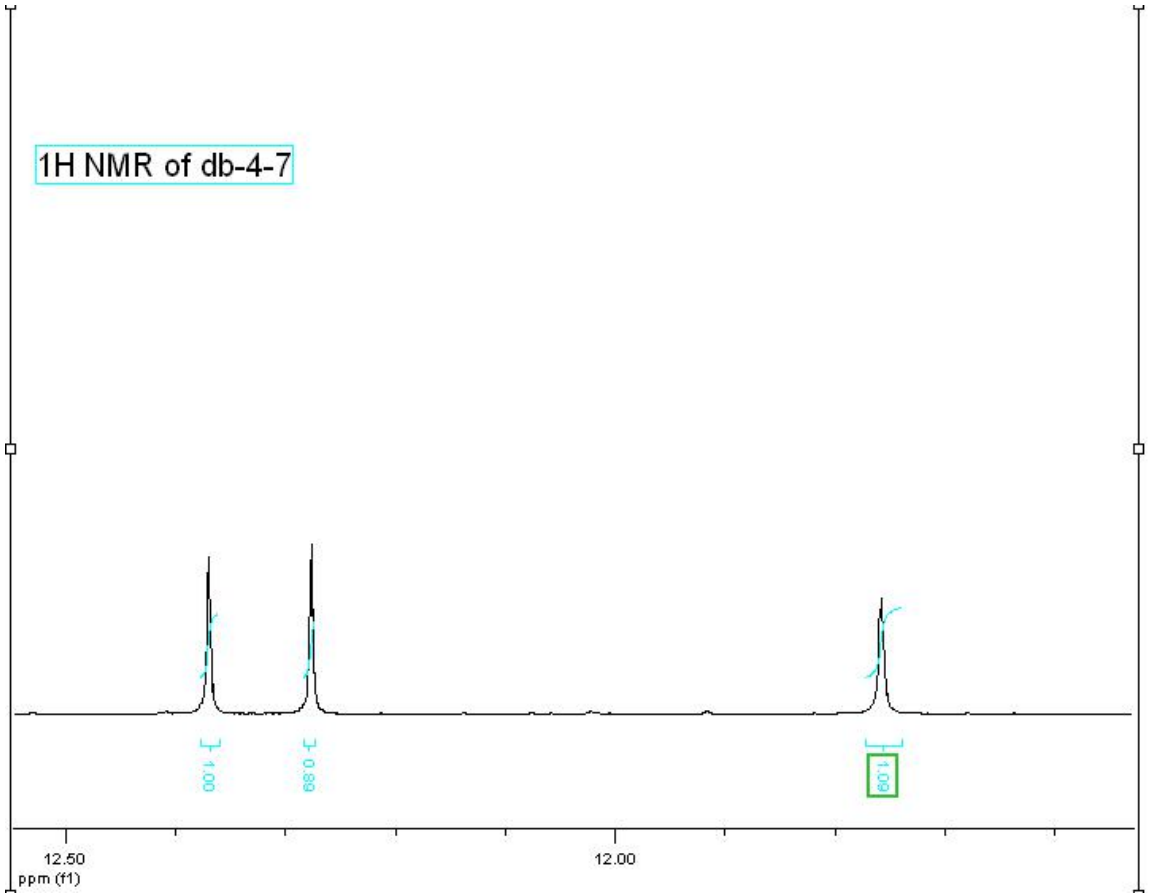
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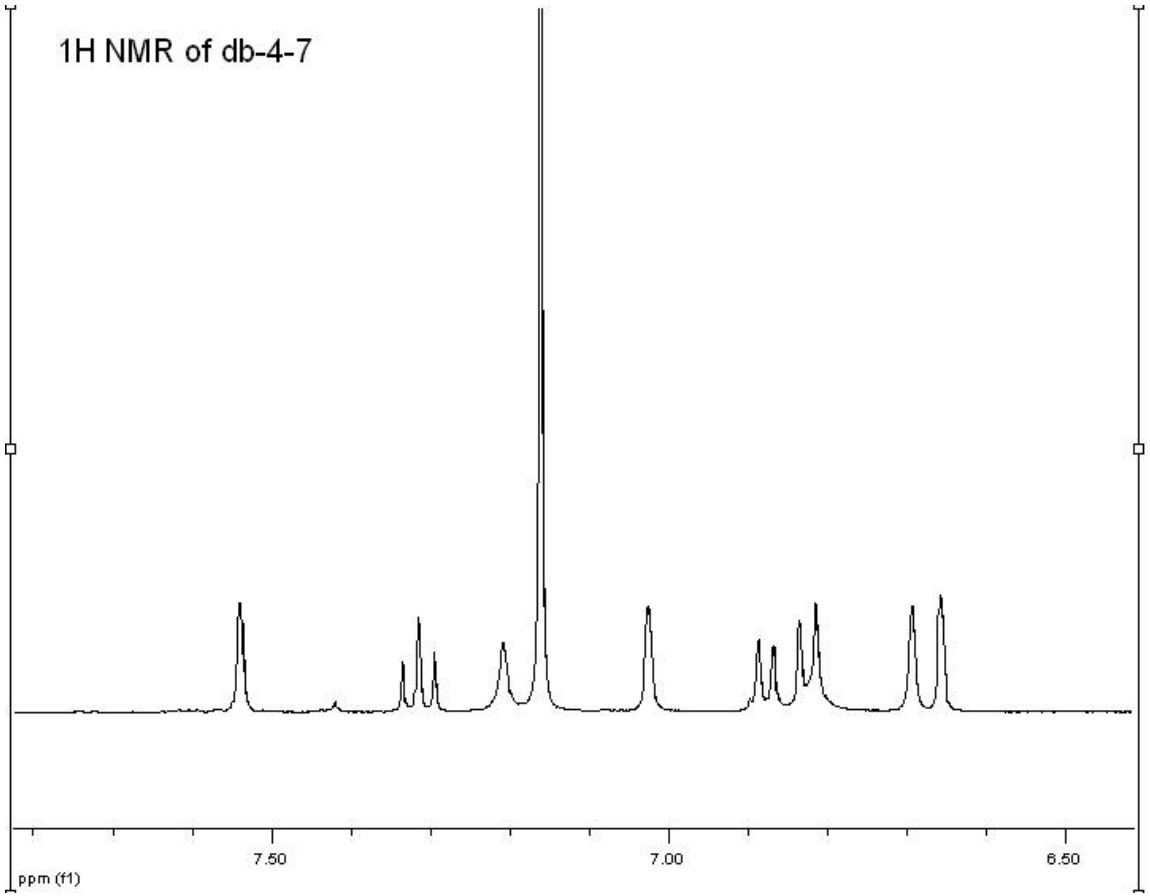
12.00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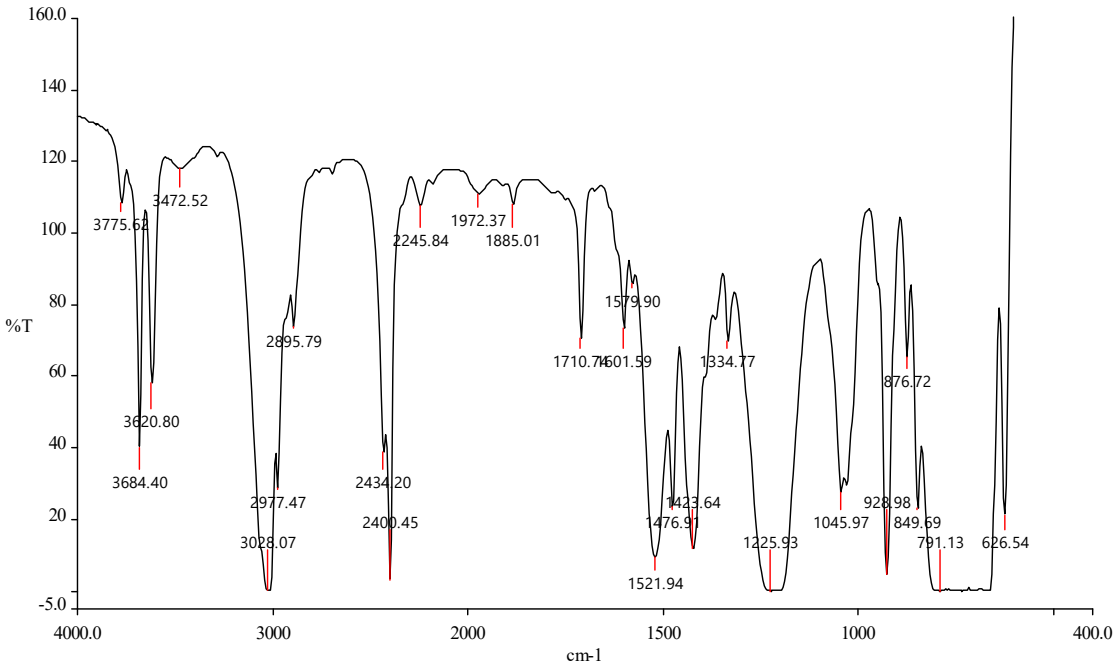
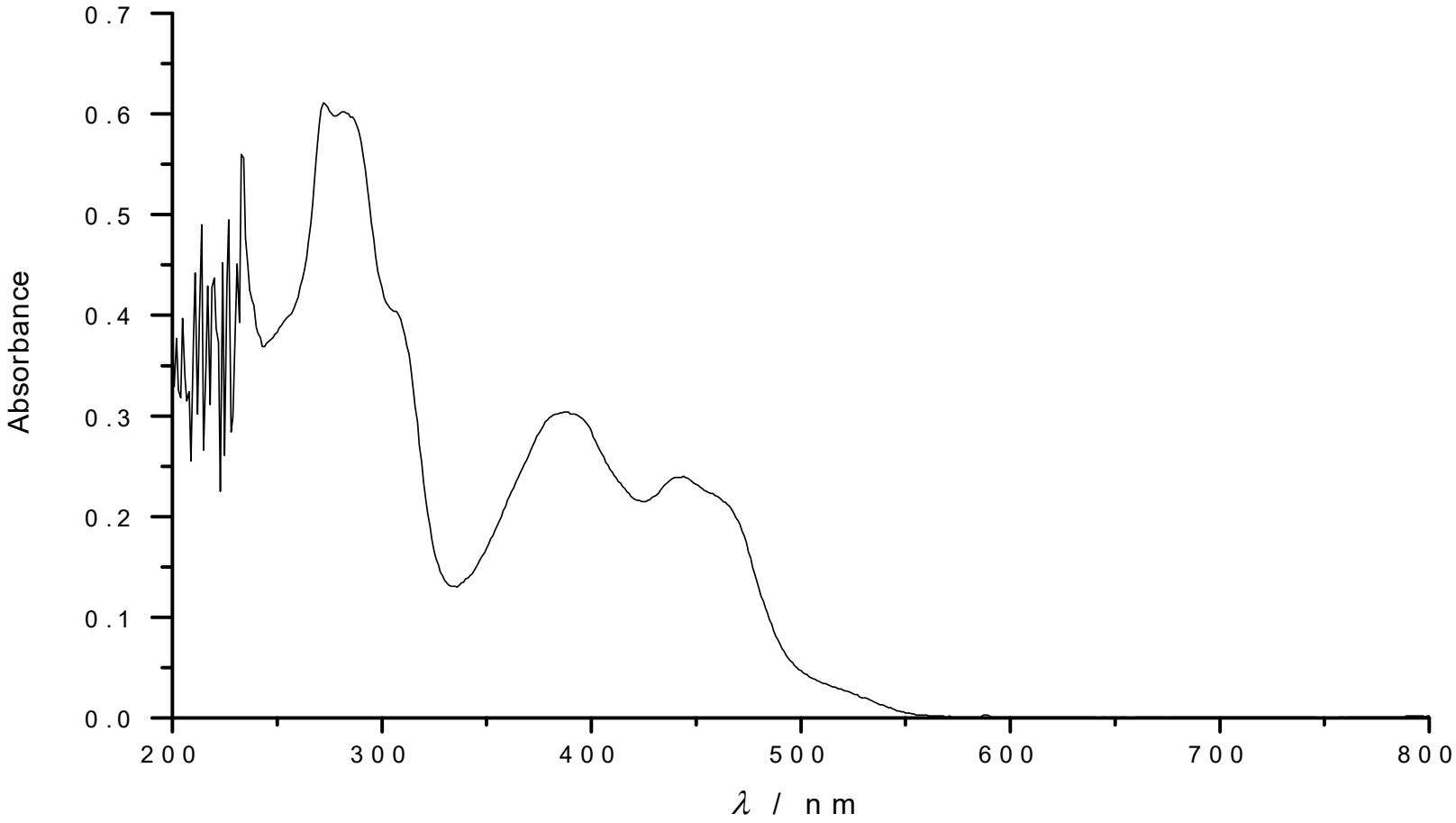
7.00
6.50
ppm (f1)



IR Spectrum of db-4-



UV-Visible Spectrum of db-4-7

Table 2. Anthraquinones isolated from Ethioian *Senna* species

Cpd No	Name of the Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Source	References
1	Chrysophanol	OH	H	Me	H	H	H	H	OH	any senna species	[22, 23]
2	Physcion	OH	H	Me	H	H	OMe	H	OH	any sema species	[22, 23]
3	Emodin	OH	H	Me	H	H	OH	H	OH	any Senna species	[22,23]
4	Aloe-emodin	OH	H	CH_2OH	H	H	H	H	OH	S. didymobotrya S. obtusfolia	[45,27, 28]
5	Rhein	OH	H	CO_2H	H	H	H	H	OH	S. didymobtrya S. alexandrina S. siama	[45, 27, 49, 50]
6	Islandicin	OH	H	Me	OH	H	H	H	OH	S. occidentalis	[23,51]
7	Helminthosporin	OH	H	Me	H	OH	H	H	OH	S. sophera S. occidentalis	[51]
8	Xanthorin	OH	H	Me	H	OH	OMe	H	OH	S. sophera S. occidentalis	[22, 51, 52]
9.	1,4,5-Trihydroxy-7-methoxy-3-methyl anthraquinone	OH	H	Me	OH	OH	H	OMe	H	S. occidentalis	[22,53]
10	Sopheranin	OH	Me	OH	H	H	OH	$CH=CH_2$	OH	S. sophera	[54]

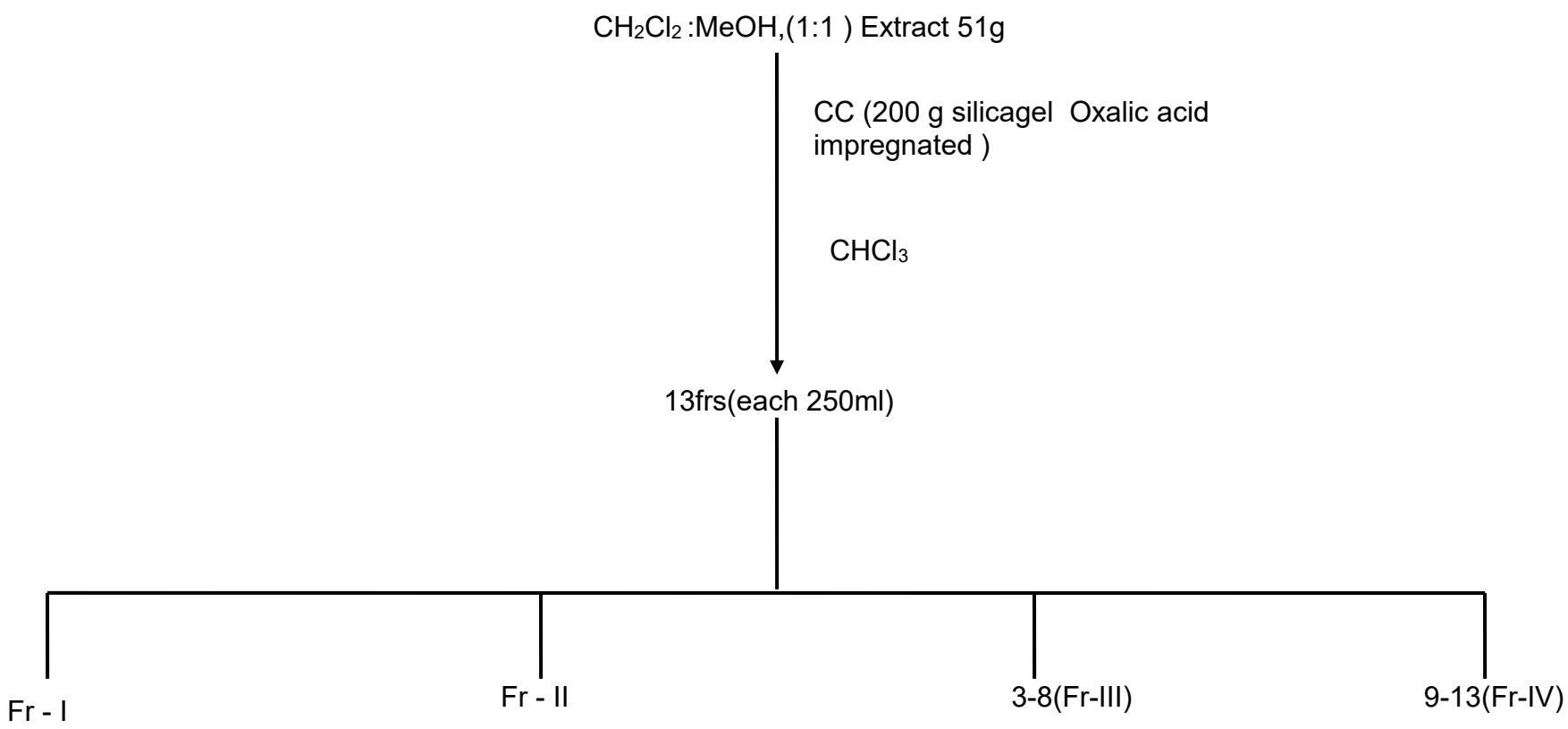
Table 2 continued…

11	1,2,7- Trihydroxy-6-8-dimethoxy-3-methyl anthraquinone	OH	OH	Me	H	H	OMe	OH	OMe	S. sophera	[54]
12	1,2,6-Trihydroxy-7,8-dimethoxy-3- methyl anthraquinone	OH	OH	Me	H	H	OH	OMe	OMe	S. sophera	[54]
13	1,3-Dihydroxy-5,7,8-trimethoxy 2-methyl anthraquinone	OH	Me	OH	H	OMe	H	OMe	OMe	S. sophera	[22,23]
14	1,8-Dihydroxy-3,6-dimethoxy-2-methyl-7-vinyl anthraquinone	OH	Me	OMe	H	H	OMe	CH_2 =CH	OH	S. sophera	[22,23]
15	2,7-Dihydroxyemodin-6,8 dimethyl ether	OH	OH	Me	H	H	OMe	OH	OMe	S. sophera	[22]
16	2,7-Dihydroxyemodin-7,8-dimethyl ether		OH	Me	H	H	OH	OMe	OMe	S. sophera	
17	1,4,8-Trihydroxy 6-methoxy-2- methyl ether	OH	Me	H	OH	H	OMe	H	OH	S. occidentalis	[23]
18	Xantorin-5- methyl ether	OH	H	Me	H	OMe	OMe	H	OH	S. sophera S. occidentalis	[23]

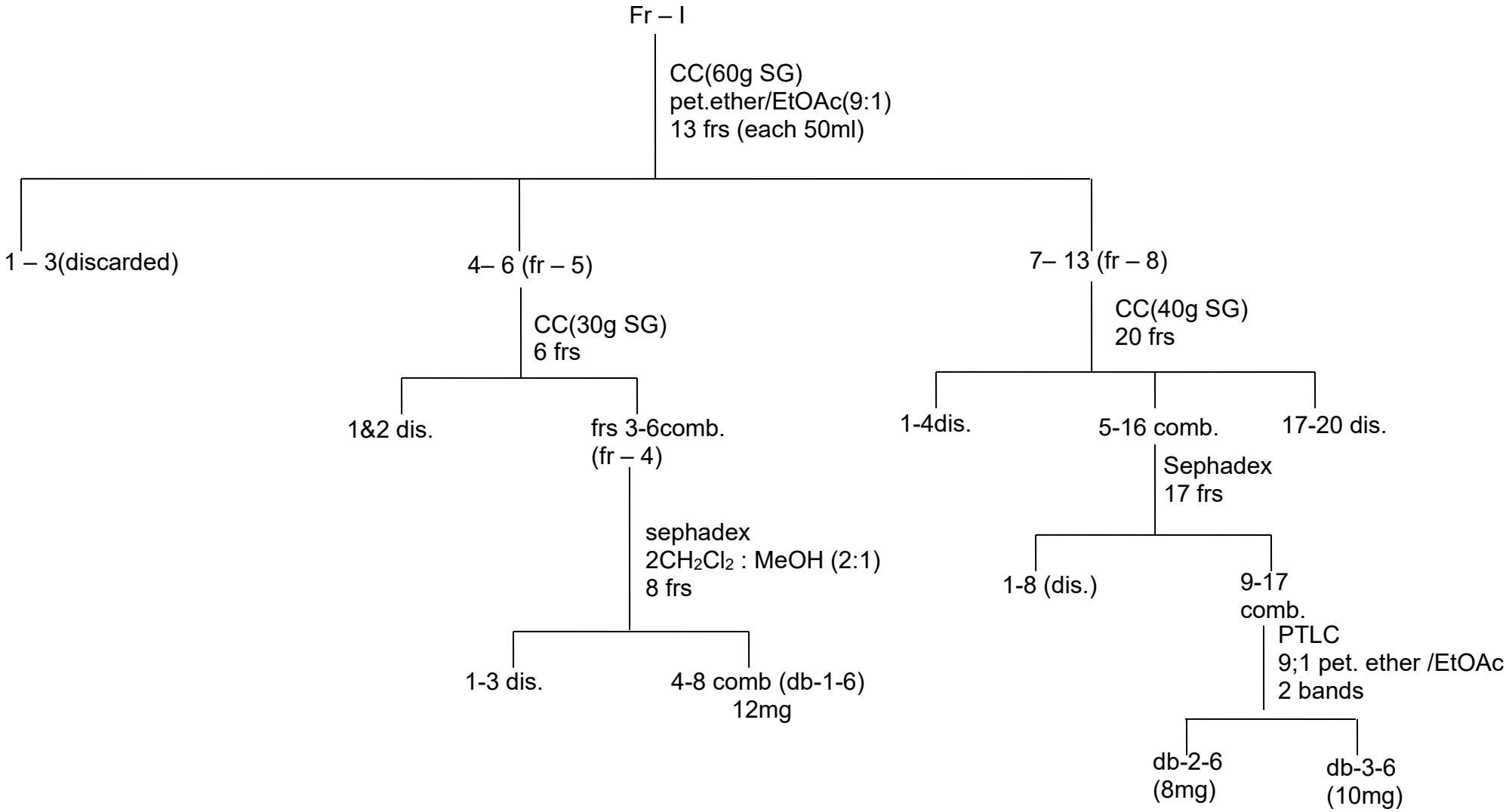
Table 2 continued…

19	Obtusin	OMe	OH	Me	H	H	OMe	OMe	OH	S. obtusfolia	[22,28, 56]
20	Auranthio-obtusin	OMe	OH	Me	H	H	OH	OMe	OH	S. obtusfolia	[22,28,56]
21	Obtusfolin	OMe	OH	Me	H	H	H	H	OH	S. obtusfolia	[22,28]
22	Chryso-obtusin	OMe	OH	Me	H	H	OMe	OMe	OMe	S. obtusfolia	[22,28,56]
23	Questin	OH	H	Me	H	H	OH	H	OMe	S. obtusfolia	[22,57]
24	1-De-O-methyl chryso-obtusin	OH	OH	Me	H	H	OMe	OMe	OMe	S. obtusfolia	[22]
25	1-De- O-methyl obtusin	OH	OH	Me	H	H	OH	OMe	OH	S. obtusfolia	[22]
26	1-De-O-methyl auranthio-obtusin	OH	OH	Me	H	H	OH	OMe	OH	S. obtusfolia	[22]
27	Chrysophanol-8-Methyl ether	OH	H	Me	H	H	H	H	OMe	S. obtusfolia	[23]
28	1,4,5-Trihydroxy-7-methyl anthraquinone	OH	H	H	OH	OH	H	Me	OH	S. occidentalis	[23,53]
29	1,8-Dihydroxy-2-methyl anthraquinone	OH	Me	H	H	H	H	H	OH	S. occidentalis	[23,53]
30	Parietenic acid	OH	H	CO_2H	H	H	OMe	H	OH	S. didymobotrya	[45]
31	Nataloe-emodin	OH	H	Me	H	H	H	OH	OH	S. longiracemosa	[58]
32	Isophyscion	OH	H	Me	H	H	H	OMe	OH	S. longiracemosa	[58]
33	7-Methyl physcion	OH	H	Me	H	H	OMe	Me	OH	S. longiracemosa	[16]

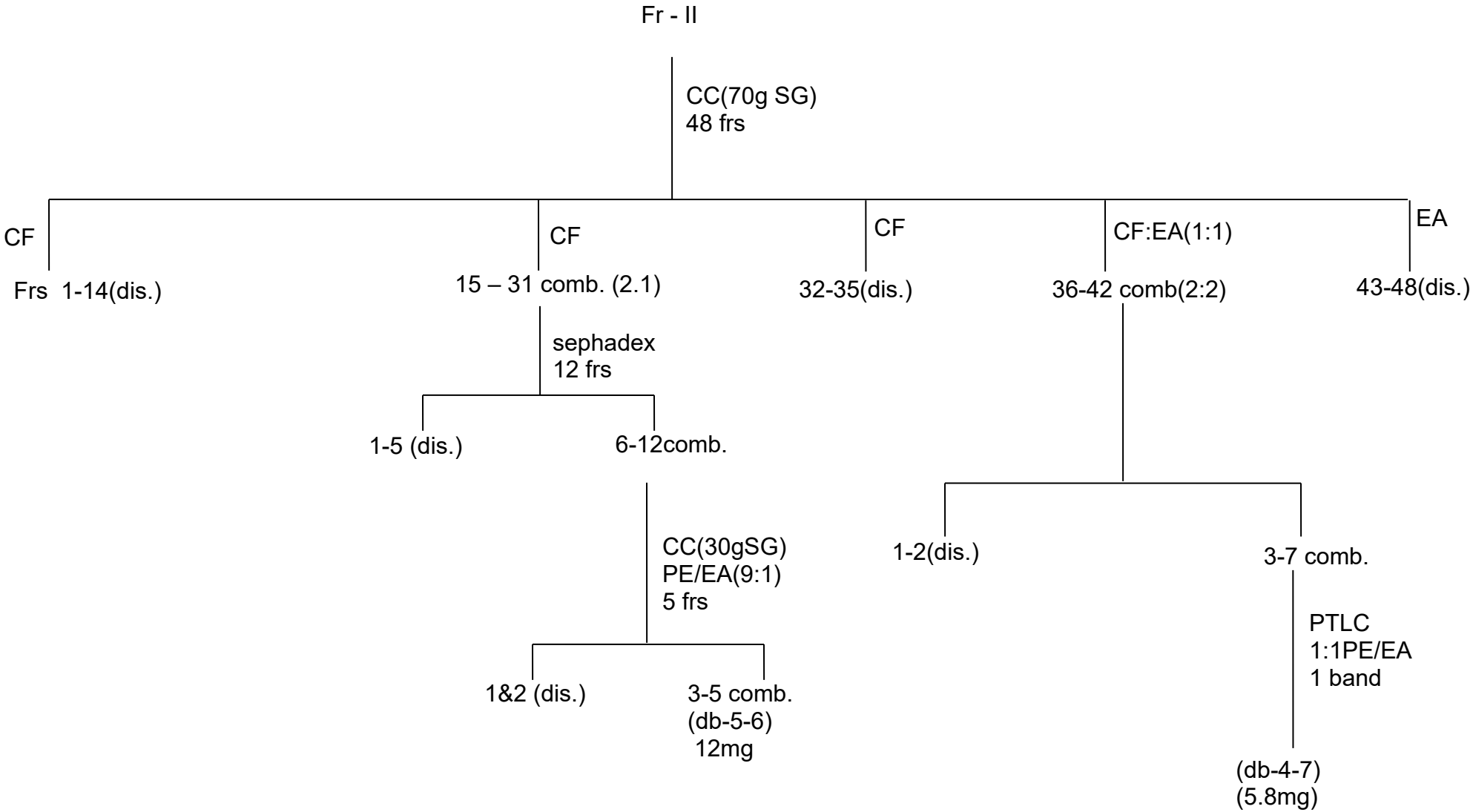
Scheme-2. cont…



Scheme-2. cont…



Scheme-2. cont…



Scheme-2. cont…