PHYTOCHEMICAL INVESTIGATION ON THE PODS OF *SENNA DIDYMOBOTRYA*

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BY
AYELE HAILU
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To my family: Meseret, Fikrete, Zenebe, Endale and Theodros Hailu.

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ABSTRACT

ANTHRAQUINONES AND FLAVONOIDS FROM THE PODS OF *S. DIDYMOBOTRYA*

Two hitherto unknown dimeric anthraquinones, 10-(Physcion-7'yl)-10-hydroxy chrysophanol anthrone (**122**) and 9-(Physcion-7'-yl)5,10-dihdroxy-2-methyl-1,4-anthraquinone (**124**) along with chrysophanol (**120**), physcion (**121**), emodin (**125**) and knipholone (**126**) were isolated from the chloroform extract of the pods of *Senna didymobotrya*. Knipholone (**126**) is isolated from the genus *Senna* for the first time.

The methanol extract of the pods yielded three flavonoids, kaempferol 4'-methyl ether (**127**), acacetin (**129**) and 3,5,6,7,8,3',4'-heptahydroxy flavone (**130**). Their presence in the genus *Senna* is reported for the first time.

Structural elucidations were carried by chemical and spectroscopic methods.

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1.0 INTRODUCTION

1.1 GENERAL

Natural products became a necessity to man since antiquity. They have been immensely utilized for various purposes, like for instance, as food additives, for the treatment of diseases, for decoration purposes, as weapons, etc..

Medicinal plants are known to provide a rich source of raw materials for traditional medicine in Africa [1], Asia [2] and other parts of the developing world, particularly those living in villages. About 85% of the people living in Africa are forced to resort to traditional practitioners and to use traditional medicine for the continued maintenance of their health and also alleviate their diverse sufferings [3]. Nature's abundant renewable supply of plants could be a great source of cheap drugs, which can be complementary to modern medicines. It is of interest to note that secondary metabolites of some plants are used as immunostimulants [4].

The study of natural products has attracted the attention and efforts of the foremost organic chemists. On the one hand these molecules of life offer challenging problems of stereospecific synthesis to the synthetic organic chemists while on the other hand their economic importance in industry, their value in folklore and modern medicine, their use in chemotaxonomy and their importance in regulating the interactions between plants and animals in nature is documented in the literature [5].

Natural products can be classified based on:

(1) Chemical structure {(i) alicyclic or cycloaliphatic compounds: e.g., terpenoids, steroids, etc., (ii) aromatic or benzenoid compounds: e.g., quinones, (iii) heterocyclic compounds: e.g., alkaloids, flavonoids},

(2) physiological activity (hormones, vitamins, antibiotics, mycotoxin), and

(3) biogenesis (acetogenin, isoprenoid, etc.).

Secondary metabolites, though there are conflicting views as to why they are produced, are believed to increase the fitness of the organism to survive. They are not useless waste or detoxification products. Rather, the ability to synthesise an array of secondary metabolites which may repel or attract other organism has evolved as one facet of the organisms' strategy for survival [7,8]. For example, flower colour and pollination, deterrent properties of some flavonoids [3] and chemical defence in termite [6] explain the usefulness of secondary metabolites for the organisms that produce them.

1.2 *SENNA* SPECIES AND THEIR MEDICINAL USES

Senna species are widely known for their medicinal value in traditional medicine (the laxative application of *S.alexandrina*, etc.). The potential of the plants in modern medicine is also exemplified by the antibacterial activity of questin isolated from *S. obtusifolia* [16] and the antimicrobial activities of torosachrysone, germichrysone, singueanol-I and singueanol-II obtained from *S. singueana* [14].

The importance of *Senna* species as a drug is compiled in Table 1.

Table 1 Medicinal uses of some *Senna* species growning in Ethiopia

plant name	Plant part used	Purpose/Remedy for	Ref.
S. didymobotrya	leaf, root, stem bark	antimalarial, purgative, gonorrhoea, cattle skin diseases, backaches, infection caused by ringworm, appetizer, antidote	9,10
S. petersiana	root, leaf,	gonorrhoea, haematuria, sterility, purgative, stomachache, syphilis anthelmintic, skin disease, coughs, syphilis	9,10,11
S. occidentalis	leaf, root	abdominal pain, snake-bite remedy, anthelmintic against round worm, oedema, fevers, malaria, antidote, stomachache, kidney troubles	9,10,12 13
S. singueana	root, bark, leaves	gonorrhoea, heartburn, purgative, stomach troubles, antibiotic	9,10,14

Table 1 (contd.)

S. sophora	seed, root	laxative, skin disease, acute bronchiate	15
S. italica	leaves, roots	laxative, gonorrhoea	12,10
S. septemtrionalis	leaves,fruits	promote menstruation, purgative	10
S. alexandrina	roots, bark, leaves	purgative	12,10
S. obtusifolia	upper parts of the plant	stomach trouble, quicken the birth, antibiotic	10,12,16
S. bicapsularis	roots	stomach complaints in children	10

1.3 ANTHRAQUINONES AND FLAVONOIDS FROM ETHIOPIAN *SENNA SPECIES*

Various types of compounds have been isolated form the different *Senna* species, including, polysaccharides, alkaloids, flavonoids, anthraquinones, glycosides of flavonoids and anthraquinones and dimers of anthraquinones. It has also been noted that anthraquinones and flavonoids are consistently present in leaves, pods, stems, roots and flowers of most species while polysaccharides are present in most of the seeds.

1.3.1 ANTHRAQUINONES FROM ETHIOPIAN *SENNA*

Anthraquinones are consistently present throughout the genus *Senna*, their presence is indicated in almost any part of these plants. Anthraquinones of the genus *Senna* are compiled in Table 2 (structures in Fig. 1).

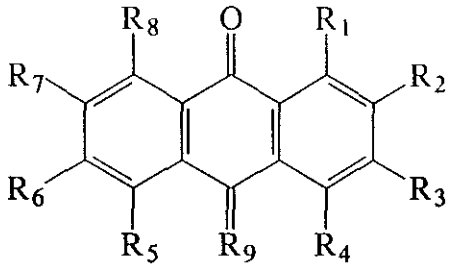


Fig. 1. List of structure of anthraquinones from *Senna*.

Structure.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
1	OH	Me	OH	H	H	OH	$CH=CH_2$	OH	O
2	OH	H	Me	H	H	H	H	OH	O
3	OH	H	Me	H	H	OMe	H	OH	O
4	OH	H	Me	H	H	OH	H	OH	O
5	OH	OH	Me	H	H	OMe	OH	OMe	O
6	OH	OH	Me	H	H	OH	OMe	OMe	O
7	OH	H	Me	H	H	H	H	OH	H,OH
8	OH	H	Me	H	H	H	H	OH	H,H
9	OMe	OH	Me	H	H	H	H	OH	O
10	OMe	OH	Me	H	H	OH	OMe	OH	O
11	OMe	OH	Me	H	H	OMe	OMe	OH	O
12	OMe	OH	Me	H	H	OMe	OMe	OMe	O
13	OH	H	Me	H	H	OH	H	OMe	O
14	OH	H	Me	OH	H	H	H	OH	O
15	OH	H	Me	H	OH	H	H	OH	O
16	OH	H	Me	H	OH	OMe	H	OH	O
17	OH	H	CH_2OH	H	H	H	H	OH	O
18	OH	H	CH_2OH	H	H	OMe	H	OH	O
19	OH	H	COOH	H	H	H	H	OH	O
20	OH	H	COOH	H	H	OMe	H	OH	O
21	OH	H	Me	H	H	H	OH	OH	O
22	OH	OH	Me	H	H	OMe	OMe	O	O
23	OH	OH	Me	H	H	OMe	OMe	OH	O
24	OH	OH	Me	H	H	OH	OMe	OH	O
25	OH	Me	OMe	H	H	OMe	$CH=CH_2$	OH	O
26	OH	Me	OH	H	OMe	H	OMe	OMe	O
27	OH	H	Me	OH	OH	H	OMe	H	O
28	OH	H	Me	H	H	OMe	Me	OH	O
29	OH	H	Me	H	H	H	H	OMe	O
30	OH	Me	H	H	H	H	H	OH	O
31	OH	Me	H	OH	H	OMe	H	OH	O

Table 2 Anthraquinones from Ethiopian *Senna*

Compound (Structure)	Isolated from	Ref.
1. Sopheranin (**1**)	***S.sophora***	17
2. 1,2,7-trihydroxy-6,8-dimethoxy-3-methyl anthraquinone (**5**)	***S.sophora***	17
3. 1,2,6-trihydroxy-7,8-dimethoxy-3-methyl anthraquinone (**6**)	***S.sophora***	18
4. Chrysophanol (**2**)	***Senna species***	
5. Physcion (**3**)	***Senna species***	
6. Emodin (**4**)	***Senna species***	
7. Chrysophanhydroanthrone (**7**)	***S. siamea***	19
8. Chrysophanol 9-anthrone (**8**)	***S. siamea***	20
9. Torosachrysone*	***S. obtusifolia***	21
	S. singueana	14
	S. didymobotrya	22
10. Germichrysone*	***S. singueana***	14
	S. occidentalis	23
11. Obtusifolin (**9**)	***S. obtusifolia***	24, 25
12. Aurantio-obtusin (**10**)	***S. obtusifolia***	24,25

* Preanthraquinone

Table 2 (contd.)

13. Obtusin (**11**)	*S. obtusifolia*	24, 25
14. Cryso-obtusin (**12**)	*S. obtusifolia*	24,25
15. Questin (**13**)	*S. obtusifolia*	26
	S. occidentalis	23
16. Islandicin (**14**)	*S. occidentalis*	27
	S. obtusifolia	25
17. Helminthosporin (**15**)	*S. occidentalis*	27
18. Xanthorin (**16**)	S. occidentalis	27
	S. obtusifolia	25
19. Aloe-emodin (**17**)	*S. occidentalis*	28
	S. didymobotrya	22
	S. obtusifolia	24,25
20. Fallacinol (**18**)	*S. didymobotrya*	22
21. Rhein (**19**)	*S. occidentalis*	28
	S. didymobotrya	22
	S. alexandrina	40
	S. siamea	20
22. Parietinic acid (**20**)	*S. didymobotrya*	22

Table 2 (contd.)

23. Nataloe-emodin (**21**)	*S. longiracemosa*	30
24. 1-De-O-methylchryso-obtusin (**22**)	*S. obtusifolia*	26
25. 1-De-O-methylobruain (**23**)	*S. obtusifolia*	26
26. 1-De-O-methylaurantio-obtusin (**24**)	*S. obtusifolia*	26
27. 1,8-Dihydroxy-3,6-dimethoxy-2-methyl-7-vinylanthraquinone (**25**)	*S. sophora*	31
28. 1,3-Dihydroxy-5,7,8-trimethoxy-2-methyl anthraquinone (**26**)	*S. sophora*	31
29. 1,4,5-Trihydroxy-7-methoxy-3-methyl anthraquinone (**27**)	*S. occidentalis*	32
30. 7-Methyl physcion (**28**)	*S. occidentalis*	26
31. Chrysophanol 8-methyl ether (**29**)	*S. obtusifolia*	26
32. 1,8-Dihydroxy-2-methyl anthraquinone (**30**)	*S. occidentalis*	32
33. 1,4,8-Trihydroxy-6-methoxy-2-methylanthraquinone (**31**)	*S. occidentalis*	33
34. 7-Methyltorosychrysone	*S. occidentalis*	23
35. Xanthone	*S. occidentalis*	23

1.3.2 BIANTHRAQUINONES FROM ETHIOPIAN *SENNA*

A number of dimeric anthraquinones have been isolated from the various *Senna* species. It is of interest to note that all the dimeric anthraquinones so far reported have only C-C linkages in between the two units. No bianthraquinones have been reported with a -C-O-C interanthraquinonoid linkage. The known dimeric anthraquinones isolated from Ethiopian *Senna species* are given in Table 3.

1.3.3 FLAVONOIDS FROM ETHIOPIAN *SENNA*

Flavonoids have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors, precursors of toxic substances, and pigments and light screens [42]. Naturally occurring flavonoids also have antiviral activity. Quercetin has been reported to possess antiviral activity against eleven types of viruses. The antiviral activity appears to be associated with non-glycosidic compounds, and hydroxylation at the 3-position is apparently a pre-requisite for antiviral activity [42].

The flavonoid pigments are responsible for the majority of flower colours present in nature and hence provide attraction to pollinating insects.

Compounds belonging to different classes of flavonoids have been implicated as insect feeding attractants, including flavonols, flavone, flavanones, dihydroflavonols, dihydrochalcones and flavanols. They also act as feeding deterrents [43].

Flavonoids from Ethiopian *Senna* are compiled in Table 4 (structures in Fig.2).

Table 3 Bianthraquinones from Ethiopian *Senna*

Compound	Isolated from	Ref.
36. Cassiamin A (2,2'-emodin-chrysophanol bianthraquinone)	*S. siamea*	34,35
	S. occidentalis	36
37. Cassiamin B (2,2'-bianthraquinone of emodin)	*S. siamea*	35
38. Cassiamin C (2.2'-bianthraquinone of chrysophanol)	*S. siamea*	35
	S. occidentalis	26
39. Siameanin (4,4'-bianthraquinone of chrysophanol	*S. siamea*	36
	S. occidentalis	26
40. Siameadin (4,4'-bianthraquinone of chrysophanol and emodin)	*S. siamea*	36
41. Cassianin	*S. siamea*	36
42. Singueanol-II	*S. singueana*	14
43. Floribudone-1 (7,5'-Biphyscion)	*S. septemtrionalis*	37
	S. multiglandulosa	38
44. Floribundone-2 (7,5'-Physcion anthrone-physcion)	*S. septemtrionalis*	37
45. 9-(Physcion-7'-yl) 5,10-dihdroxy-7-methoxy-2-methyl-1, 4-anthraquinone	S. multiglandulosa	38
46. Torosanin-9'-10'-quinone	*S. multiglandulosa*	38
	S. septemtrionalis	39

Table 3 (contd.)

47. Anhydrophlegmacin-9',10'-quinone	*S. septemtrionalis*	39
	S. multiglandulosa	38
48. Palmidin D (chrysophanol-physcion 10,10'-bianthrone	*S. occidentalis*	36
	S. alexandrian	40
49. Aloe-emodin-10,10'-bianthrone	*S. alexandrian*	40
50. 10,10'-Chrysophanol-isophyscion	*S. longiracemosa*	30
51. Physcion-10,10'-bianthrone	*S. longiracemosa*	30
52. 10-Hydroxy-10,7'-(chrysophanol-anthrone)-chrysophanol	*S. longiracemosa*	30
53. 10,10'-Bichrysophanol	*S. longiracemosa*	30
54. 10,10'-Chrysphanol-physcion	*S. longiracemosa*	30
55. Chrysophanol-dianthrone	*S. alexandrian*	40
	S. siamea	20
56. Rheidin B (rhein-chrysophanol-bianthrone)	*S. alexandrian*	40
57. Palmidin C (emodin-chrysophanol bianthrone)	*S. alexandrian*	40
58. Palmidin B (aloe-emodin-chrysop-hanol-bianthrone)	*S. alexandrian*	40
59. Rheidin A (rhein-emodin-bianthrone)	*S. alexandrian*	40
60. Emodin-dianthrone	*S. alexandrian*	40
61. Palmidin A (aloe-emodin-emodin bianthrone)	*S. alexandrian*	40

Table 3 (contd.)

62. 4,4`,5,5'-Tetrahydroxy-2,2'-dimethyl,1,1'-bianthraquinone	*S. occidentalis*	41
63. Occidentalol-I	*S. occidentalis*	23
64. Occidentalol-II	*S. occidentalis*	23
65. Singueanol-I	*S. occidentalis*	23
	S. singueana	14

Table 4 Flavonoids from Ethiopian *Senna*

Compound (structure)	Isolated from	Ref.
66. Quercetin (**66**)	*S. obtusifolia*	44
	S. sophora	18
	S. italica	45
	S. septemtrionalis	46,47
67. Ombuin (**67**)	*S. septemtrionalis*	48,49
68. Apigenin (**68**)	*S. siamea*	51
	S. italica	45
69. Kaempferol (**69**)	*S. italica*	45
	S. siamea	51
	S. obtusifolia	44
	S. alexandrian	52
70. Apigenin-5,7,4'-trimethyl ether (**70**)	*S. siamea*	33

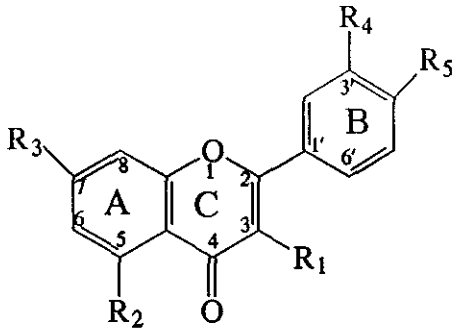


Fig.2. List of structures of flavonoids from Ethiopian *Senna*

	R_1	R_2	R_3	R_4	R_5	
66.	OH	OH	OH	OH	OH	Quercetin
67.	OH	OH	OMe	OH	OMe	Ombuin
68.	H	OH	OH	H	OH	Apigenin
69.	OH	OH	OH	H	OH	Kaempferol
70.	H	OMe	OMe	H	OMe	Apigenin 5,7,4'-trimethyl ether

1.3.4 GLYCOSIDES FROM ETHIOPIAN *SENNA*

1.3.4.1 ANTHRAQUINONE GLYCOSIDES

The presence of sugar moiety in anthraquinone and also the type of sugar, though presumably has no direct activity of its own, is important for the enhancement of pharmacological activities of the aglycone [53]. This is because the glycosides are soluble in body fluids such that they pass via the blood stream to the site of action (large intestine).

Anthraquinones are prone to metabolic detoxification. The presence of the sugar group confers a resistance on the glycosides such that it withstands this detoxification mechanism of the body [53]. Anthraquinone glycosides of the genus *Senna* are given in Table 5.

Table 5 Anthraquinone glycosides from Ethiopian *Senna*

Compound	Isolated from	Ref.
71. Emodin 8-O-sophoroside	*S. alexandrina*	54
72. Aloe-emodin 8-O-glucoside	*S. alexandrina*	54
73. Aloe-emodin dianthrone 8,8'-di-O-glucoside	*S. alexandrina*	54
74. Sennosides A & B (birhein-9-anthrone-8,8'-diglucoside)	*S. alexandrina*	40,55,56
75. Sennosides C & D (aloe-emodin-rhein bianthrone-8,8-diglucoside)	*S. alexandrina*	40
76. Physcion 8-O-galactose	*S. septemtrionalis*	48
77. Physcion-8-O-digalactoside	*S. septemtrionalis*	57
78. Rhein-8-O-glucoside	*S. alexandrina*	58
79. Rhein-8-diglucoside	*S. alexandrina*	58
80. Rhien anthrone-8-glucoside	*S. alexandrina*	58
81. Aloe-emodin glucoside	*S. alexandrina*	58
82. Chrysophanic acid glycoside	*S. alexandrina*	59
83. Physcion 1-β-D glucopyranoside	*S. occidentalis*	60
84. Sennoside A_1(-)-sennidin 8,8'-diglucoside	*S. alexandrina*	61
85. Aloe-emodin anthrone monoglucoside	*S. alexandrina*	62

Table 5 (contd.)

86. Aloe-emodin anthrone diglucoside	*S. alexandrina*	62
87. Emodin athrone monoglucoside	*S. alexandrina*	62
88. Chrysophanol anthrone diglucoside	*S. alexandrina*	62
89. Physcion anthrone diglucoside	*S. alexandrina*	62
90. Rhein anthrone diglucoside	*S. alexandrina*	62
91. Obtusifolin mono-β-D-glucoside	*S. obtusifolia*	63
92. Aurantioobtusin 6-monoglucoside	*S. obtusifolia*	63

1.3.4.2 FLAVONOID GLYCOSIDES

Flavonoid glycosides are as abundant as their anthraquinone counterparts in the *Senna species*. They are compiled in Table 6.

1.4 BIOSYNTHESIS OF ANTHRAQUINONES

Generally two different biogenetic routes have been suggested for the formation of quinones in nature [36].

i) the shikimic acid route,

ii) the polyacetate route

Since the anthraquinones in genus senna have substituents in both the benzenoid rings, the polyketide origin is very plausible [72-74].

1.5 BIOSYNTHESIS OF FLAVONOIDS

The flavonoid variants are all related by a common biosynthetic pathway which incorporates precursors from both the "Shikimate" and "Acetate-Malonate" pathways [75]. The flavonoid initially formed in the biosynthesis is now believed to be the chalcone [76] and all other forms are derived from this by a variety of routes. Further modification of the flavonoid may occur at various stages resulting in: additional (or reduced) hydroxylation; methylation of hydroxyl groups or of the flavonoid nucleus; isoprenylation of hydroxyl groups or of the flavonoid nucleus; dimerization (to produce biflavonoids); and most importantly, glycosylation of hydroxyl groups (to produce *flavonoid O-glycosides*) or of the flavonoid nucleus (to produce *flavonoid C-glycosides*).

Table 6 Flavonoid glycosides from Ethiopian *Senna*

Compound	Isolated from	Ref.
93. Matteucinol 7-rhamonoside	*S. occidentalis*	64
94. Jaceidin 7-rhamnoside	*S. occidentalis*	64
95. Quercetin 7,4-dimethyl-ether 3-galactoside	*S. septemtrionalis*	65
96. Quercetin 7,3',4'-trimethylether	*S. septemtrionalis*	65
97. Quercetin 7,4'-dimethyl ether (ombuin) 3-Neohesperidoside	*S. septemtrionalis*	66
98. Rhamnetin digalactoside (3-galactosyl (1→4)-galactopyranoside	*S. septemtrionalis*	67
99. Rhamnetin digalactoside (3-galactosyl (1→6) galactopyranoside	*S. septemtrionalis*	67
100. Quercetin 3,7-di-rhamnopyranoside	*S. septemtrionalis*	68
101. 3',5-Dihydroxy-4',7-dimethoxy flvone 3-O-β-D(+)-glucopyranoside	*S. septemtrionalis*	60
102. 5-Hydroxy-3',4',7-trimethoxy flvone 3-O-β-D(+)-galactosyl-O-β-D(+)-galactopyranoside	*S. septemtrionalis*	60
103. Quercitrin	*S. obtusifolia*	44
104. Isoquercitrin	*S. obtusifolia*	44
	S. didymobotrya	69
105. Thalictiin (apigenin-7-O-galactoside)	*S. siamea*	70
106. Kaempferol-3-rhamnoside	*S.didymobotrya*	69

Table 6 (contd.)

107. 8-C-Rhamnosyl europetin (3,5,3',4',5'- pentahydroxy 7-methyl ether 8-rhamnoside)	*S. sophora*	29
108. Tamarixetin 3-rutinoside-7-rhamnoside	*S. italica*	45
109. Apigenin 7-glucoside	*S. italica*	45
110. Kaempferol 7-glucoside	*S. italica*	45
111. Quercetin 7-glucoside	*S. italica*	45
112. Kaempferol 3-rutinoside	*S. italica*	45
113. Quercetin 3-rutinoside	*S. italica*	45
114. Isorhamnetin-3-rutinoside-7-rhamnoside	*S. italica*	45
115. Rhamentin-3-O-β-Diglycoside	*S. sophora*	71
116. Kaempferol 3-O-gentiobioside{β-D-glucopyranosyl-(1→6)- β-D-glucopyranoside}	S. alexandrina	54
117. Quercetin 3-O-gentiobiosides	*S. alexandrina*	54
118. Isorhamnetin 3-O-gentiobiocides	*S. alexandrina*	54
119. Jaceidin 7-O-neohesperidoside (ombuin) 3-digalactoside	*S. occidentalis*	33

1.6 IDENTIFICATION OF FLAVONOIDS AND ANTHRAQUINONES

1.6.1 FLAVONOIDS

By spraying the chromatogram with different reagents, a limited amount of information about the structure of flavonoid can be gained [45 & 75]. The orientation of hydroxyl group in the flavonoid skeleton can be determined from UV spectrum using shift reagent [45, 75, 85 & 86]. The use of ^{1}H-NMR and ^{13}C-NMR for structure elucidation are very well documented in literature [75, 87 & 92].

1.6.1 ANTHRAQUINONES

Anthraquinones can be identified on the basis of their colour reaction with alkaline and alcoholic magnesium acetate solution by spraying on the chromatogram [36 & 77] as well as spectroscopic evidences (UV [36, 50, 78-81], IR [36, 82 & 83] ^{1}H-NMR [36]).

The location of O-alkyl and O-glycosyl substituents in 1,8-dihydroxy anthraquinones is a common structural problem. Kalidhar [84] has found a solution by comparison of the ^{1}H-NMR data of the anthraquinone aglycone with that of anthraquinone-O-glycosides and anthraquinone methyl ether of the same basic skeleton.

Comparison of the ^{1}H-NMR data of the peracetates of emodin and emodin-8-O-glucoside shows that H-5, the proton which is para to the site of glycosylation, undergoes the most significant change in chemical shift (Tables 7 & 8). This change has been measured by the glycosylation shifts of the proton (ΔH), i.e., the difference between the chemical shifts of the proton in an anthraquinone aglycone peracetate and its glycoside. As is evident from Table 7, the signal of H-5 is greatest when the glycoside is at position 8. Similar behaviour has been observed for 8-methylether and the shift has been denoted by Δ'H-5. In emodin-1-O-glucoside the largest chemical shift is that of H-4, which is para to the position of glycosylation.

If there is a glycoside at position 6 in emodin, it is also possible to ascertain this position with the help of the proposed method. There is no aromatic proton para to the site of glycosylation but it has been observed that the signal of the two protons ortho to the position of the glycoside, i.e., the chemical shifts of H-5 and H-7, undergo by

more than 0.2 and a similar trend has been observed for 6-O-alkylation.

Consequently, for a glycoside, if ΔH-5 gives the highest value then 8-O-glycosylation (or alkylation) is indicated and when ΔH-5 and ΔH-7 are both more than 0.2, 6-O-glycosylation (alkylation) is expected. A large value of ΔH-4 suggests 1-O-glycosylation (alkylation).

Table 7: Chemical shift (in $CDCl_3$) of aromatic protons in the ^{1}H-NMR spectrum of the peracetates of emodin and related compounds [84]

	Chemical shift			
Compound	H-2 brs	H-4 brs	H-5 d, J=2.5 Hz	H-7 d, J=2.5 Hz
Emodin	7.24	8.03	7.98	7.26
Emodin-8-O-glucoside	7.23	7.97	7.76	7.31
Emodin-6-O-glucoside	7.19	7.91	7.54	6.98
Physcion	7.24	8.03	7.69	6.92

Table 8: Glycosylation(ΔH)* or alkylation (Δ'H)+ shifts in emodin and related compounds [84]

Compound	ΔH-2 (Δ'H-2)	ΔH-4 (Δ'H-4)	ΔH-5 (Δ'H-5)	ΔH-7 (Δ'H-7)
	8-O-Glycosylation.			
Emodin-Emodin-8-O-glucoside	+0.01	+0.06	+0.22	-0.05
	6-O-Glycosylation.			
Emodin-Emodin-6-O-glucoside	+0.05	+0.12	+0.44	+0.28
	6-O-Alkylation.			
Emodin-Physcion	(0.0)	(0.0)	(+0.29)	(+0.34)

ΔH = δ value of the aromatic proton in aglycone peracetate minus that in O-glycosyl peracetate.
+Δ'H = δ value of the aromatic proton in anthraquinone peracetate minus that in O-alkyl peracetate.

1.7 OBJECTIVE

The genus *Senna* which belongs to the Leguminosae family has about 240 species mainly found in the tropical and subtropical zones of the world [12]. In Ethiopia, there are eighteen species belonging to the genus [12]. They are (synonyms are indicated in brackets), *Senna petersiana* (*Cassia petersiana*), *S. septemtrionalis* (*C. septemtrionalis*, *C. laevigata*, *C. floribunda*), *S. singueana* (*C. singueana*, *C. sabak*, *C. goratensis*), *S. baccarinii*, (*C. baccrinii*), *S. occidentalis*, (*C. occidentalis*), *S. sophora*, (*C. sophora*) *S. obtusifolia*, (*C. obtusifolia*, *C. tora*), *S.siamea* (*C.siamea*), *S.ellisiae*, (*C. ellisiae*), *S. longiracemosa*, (*C. longiracemosa*), *S. ruspolii*, (*C. ruspolii*), *S. didymobotrya*, (*C. didymobotrya*), *S. truncata*, (*C. truncata*), *S. italica*, (*C. italica*), *S. holosericea*, (*C. holosericea*), *S. multiglandulosa*, (*C. multiglandulosa*, *C. tomentosa*), *S. alexandrina*, (*C. alexandrina*, *C. senna*, *C. angustifolia*), *S. bicapsularis*, (*C. bicapsularis*).

The objective of this project is isolation and structural elucidation of secondary metabolites, i.e., anthraquinones and flavonoids from the pods of *S. didymobotrya*.

S. didymobotrya is a bushy shrub about 3-4 m high or occasionally a tree up to 6 m high, leaves up to 0.5 m long, common from 1450 to 2450 m in good rainfall areas [12, 88]. In Ethiopia, it is found in Wello, Welega, Shewa, Sidamo and Arsi regions [12].

It has a number of applications in traditional medicine. A decoction of the leaves, stems and roots have been used for the treatment of sexually transmitted disease (like gonorrhoea), cattle diseases, backaches and it is also used as a purgative and appetizer [10].

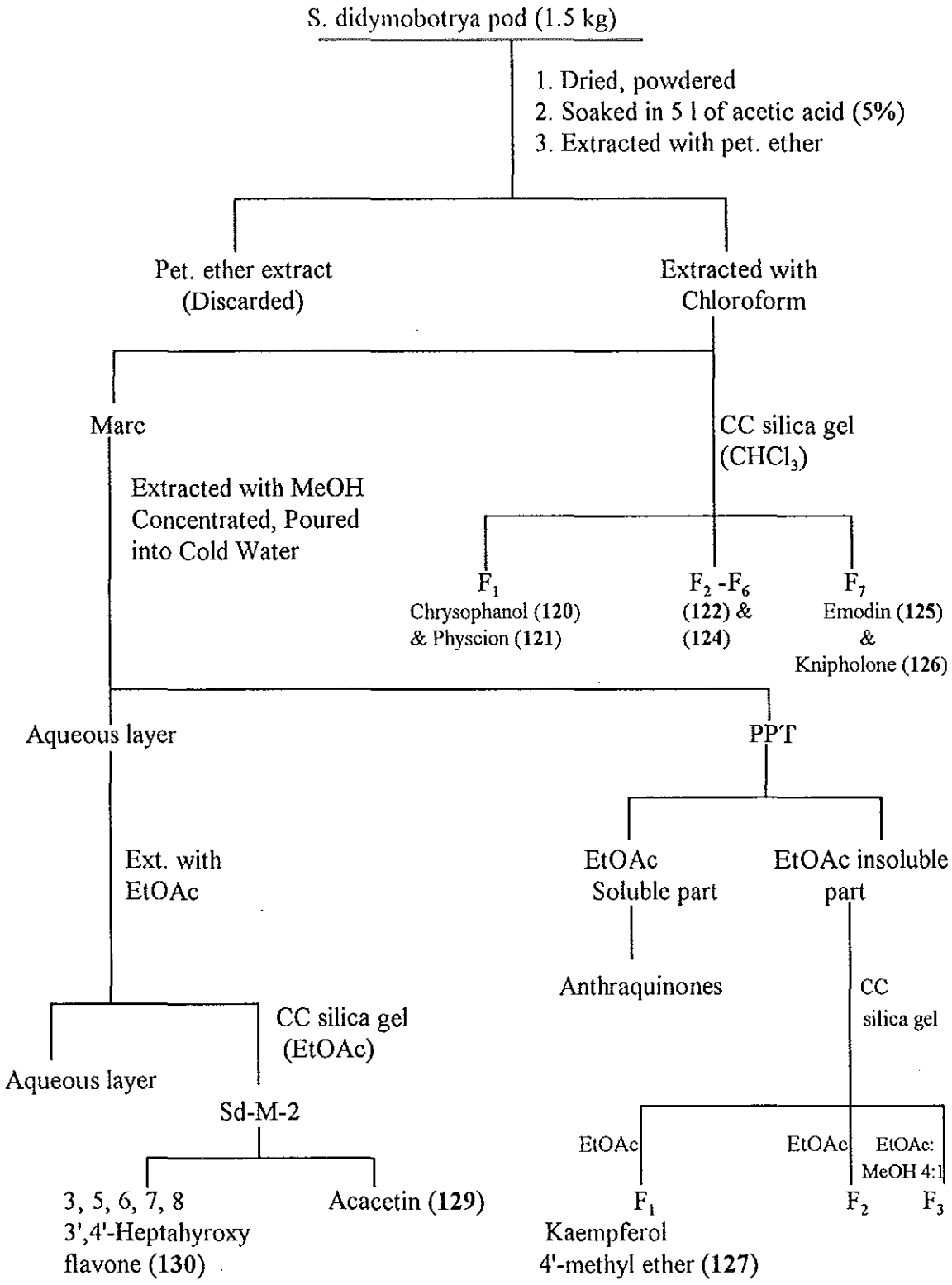
Previous phytochemical investigations on the leaves of the plant led to the isolation of chrysophanol, aloe-emodin, rhein, physcion, fallacinol, parietenic acid, torosachrysone kaempferol-3-rhamnoside and isoquerctin [27,69]. There is no literature report on the chemical constituents of the pods of this plant prior to this work. In view of this, the chemical investigation of the pods of *S. didymobotrya* has been undertaken.

2.0 RESULTS AND DISCUSSION

In this study we have examined the seeds and pods of *S. didymobotrya*. The chloroform extract of the seeds when developed on TLC and sprayed with 5% methanolic KOH did not show characteristic colour for anthraquinones. In contrast to the seeds, the chloroform extract of the pod indicated positive tests for anthraquinones while the methanol extract indicated positive tests for both anthraquinones and flavonoids. So we turned our attention to the chemical investigation of the pods.

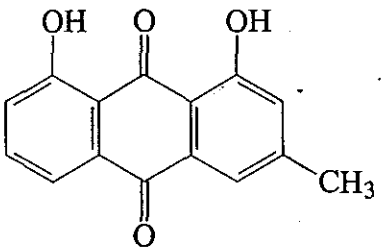
As shown in the following scheme, used to partition the phenolic constituents, six anthraquinones and three flavonoids were isolated and their structures were elucidated from analysis of their ^{1}H-NMR, UV, IR and MS spectral data.

SCHEME: METHOD USED TO PARTITION ANTHRAQUINONES FROM FLAVONOIDS

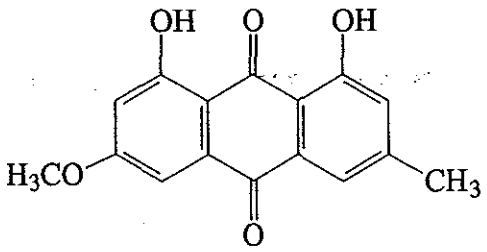


2.1 THE CHLOROFORM EXTRACT

The chloroform extract (see the scheme) was chromatographed into seven fractions (F_1-F_7). The first fraction gave the common anthraquinones chrysophanol (120) and physcion (121). The compounds were identified by comparison with authentic samples (TLC) and on the basis of their spectroscopic data (^{1}H-NMR, UV and IR) which agree with those reported in the literature [36, 89].



Chrysophanol (120)



Physcion (121)

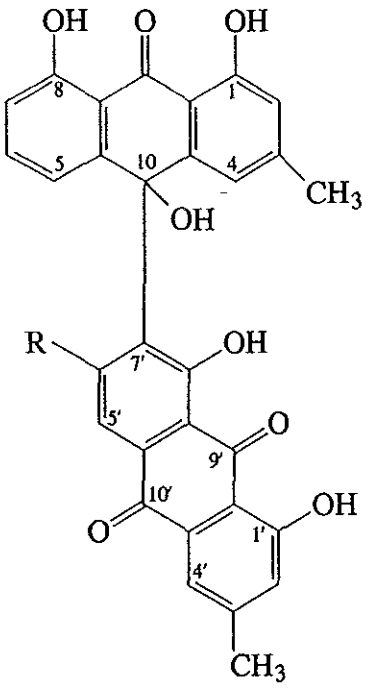
On the basis of TLC, fractions F_2-F_6 were combined. The combined fraction which showed two major spots on TLC (Silica gel, $CHCl_3$) with R_f of 0.36 and 0.26 was separated on PTLC (Silica gel, $CHCl_3$) to give 122 and 124 .

10-(Physcion-7'yl)-10-hydroxy chrysophanol anthrone (122)

10-(Physicon-7'yl)-10-hydroxy chrysophanol anthrone (**122**), was obtained as an orange pigment, mp 182-184 °C. The similarity in the chromophore of **122** and **123**, which was isolated from *Senna longiracemosa* [30], was established by comparison of their UV and IR spectra. The absorptions at 224, 271, 303, 384, and 468 nm showed that **122** possessed both beneznoid and quinonoid chromophores, while bands at 3428, 1667, 1616 $^{-1}$cm revealed the presence of hydroxyl, unchelated and chelated carbonyl groups, respectively. The ^{1}H-NMR spectrum of **122** showed the presence of two aromatic methyls, a methoxy and eight aromatic protons, as well as four chelated hydroxyl groups. A comparison of the ^{1}H-NMR of **122** with that of **123** shows that **122** has one less aromatic proton (δ 7.98) and one additional methoxy group (δ 3.90) than that of **123** (see Table 11). The mass spectrum (MS) of **122** gave molecular formula $C_{31}H_{22}O_9$ (m/z 538 $[M]^+$) which accounts for an anthraquinone based on chrysophanol and physcion moieties. The presence of the two moieties was confirmed from the MS which showed fragment ions at m/z 284 and 255, corresponding to physcion and chrysophanol moieties, respectively.

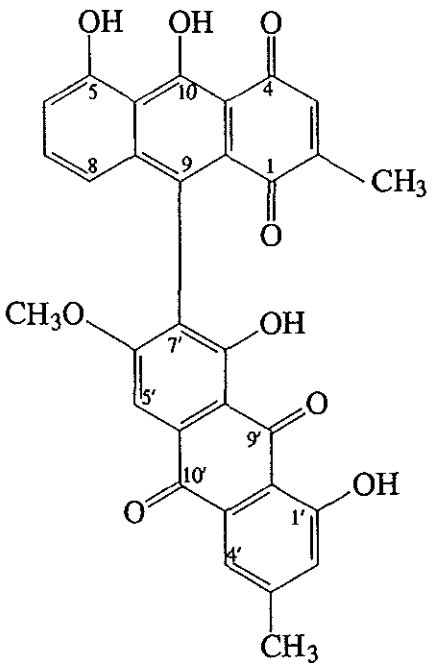
The two meta coupled protons at δ 7.01 (brs, H-2) and 6.80 (brs, H-4) together with an ABC system at δ 6.95 (d, J=8.0 Hz), 7.55 (t, J=8.0 Hz) and 7.12 (d, J=8.0 Hz) for H-5, H-6 and H-7, respectively are assignable to the protons of the chrysophanol moiety. The signals at δ 7.21 (brs), 7.64 (brs) and 7.40 (s) are attributed to H-2, H-4 and H-5, respectively, of the physcion moiety.

The upfield signals for H-4 (δ 6.80) and H-5 (δ 6.95) indicate the anthrone nature of the chrysophanol moiety. This indicates that the attachment of the physcion moiety is on C-10 of the chrysophanol skeleton. The signal for H-7 (δ 6.50) of physcion has disappeared in the spectrum of **122** which indicated that the chrysophanol moiety is coupled on C-7 position of the physcion moiety. Therefore the two monomeric units are linked at C-10 of chrysophanol and C-7 of the physcion moieties.



122: R = OCH_3

123: R = H



124

Table 9: ^{1}H-NMR spectral data of **122** (300 MHz, acetone-d_6) and **123** (400 MHz, $CHCl_3$) [30].

H	**122** δ/ppm	**123** δ/ppm
1-OH*	12.30 s	11.75 s
2	7.01 brs	6.76 d (J=2.0 Hz)
3-Me	2.30 s	2.20 s
4	6.80 brs	6.58 d (J=2.0 Hz)
5	6.95 d (J=8.0 Hz)	6.78 dd (J=7.8, 2.0 Hz)
6	7.55 t (J=8.0 Hz)	7.38 t (J=7.8)
7	7.12 d (J=8.0 Hz)	6.94 dd (J=7.8, 2.0 Hz)
8-OH*	12.32 s	12.15 s
1'-OH*	12.38 s	12.35 s
2'	7.21 brs	7.02 d (J=2.0 Hz)
3'-Me	2.50 s	2.40 s
4'	7.64 brs	7.60 d (J=2.0 Hz)
5'	7.40 s	8.64 d (J=7.8 Hz)
6'-OMe	3.80 s	
6'		7.98 d (J=7.8 Hz)
8'-OH*	12.40 s	12.45 s

*: Signals may interchange

9-(Physcion-7'yl)5,10-dihydroxy-2-methyl-1,4-anthraquinone (124)

9-(Physcion-7'yl)5,10-dihydroxy-2-methyl-1,4-anthraquinone (**124**) is a brown pigment, which turned pink when sprayed with 5% methanolic KOH. The UV spectrum has absorption maxima at 239, 300, 446, 500, 536 and 580 nm suggesting the presence of a quinonoid chromophore. The IR spectrum showed bands for a hydroxyl group at 3452 cm^{-1}, a free and a chelated carbonyl at 1731 and 1626 cm^{-1}, respectively. Its ^{1}H-NMR data revealed seven aromatic protons, two methyl groups, a methoxy and four hydroxyl groups which can be accommodated on a bianthraquinone skeleton. The two hydroxyl groups at δ 12.05, and 12.25, a methoxy at δ 3.84 together with three aromatic protons signals at δ 7.62 (s), 7.70 (brs) and 7.15 (brs) ppm are assigned to the physcion moiety with the aromatic signals belonging to H-5, H-4, and H-2 respectively.

The non-physcion moiety contains chelated and doubly chelated OHs resonating at δ 10.6 and 17.1, respectively, three aromatic protons with an ABC splitting pattern, a methyl group and one Q-H which suggests the presence of a 1,4-quinone structure. This is supported by the UV-Vis absorbtion which showed a bathochromic shift of its long wavelength absorbtion maximum and a low molar extinction coefficient typical of 1,4-anthraquinones. The three aromatic signals at δ 7.12, 7.50 and 6.98 ppm are assignable to the 1,4-quinone moiety with the Ar-H signals belonging to H-6, H-7 and H-8, respectively. The Q-H and the methyl groups resonate at δ 6.95 and 2.10, respectively. These indicate that the 1,4-quinone is 5,10-dihydroxy-2-methyl-1,4-quinone. The signal attributable to 9-H, which resonates relatively downfield, of the 1,4-quinone moiety is missing, suggesting the coupling of the physcion moiety to be at position 9 of the 1,4-quinone skeleton.

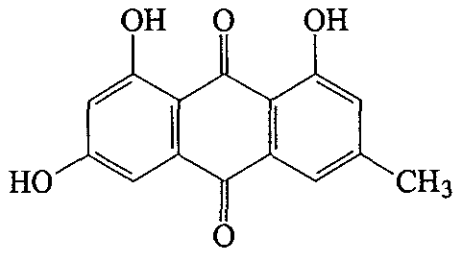
All the signals of the physcion moiety except that of H-7 were clearly observed on the spectrum of 124 and closely match with that of physcion. This indicates that the 1,4-quinone is linked with C-7 of the physcion moiety.

The MS spectrum indicates the molecular ion peak $[M]^+$ at 536 corresponding to a molecular formula of $C_{31}H_{20}O_9$ which is in accord to the proposed structure (**124**).

The seventh fraction was freed from green pigments by Sephadex (LH-20, $CHCl_3$:MeOH 2:1) and two chlorophyll free fractions were obtained. The second fraction, which contained two major spots, was purified on PTLC (Silica gel, pet. ether: EtOAc 4:1) to give emodin (**125**) and knipholone (**126**).

Emodin (125)

^{1}H-NMR spectrum of **125** indicates the presence of two chelated hydroxyl groups at δ 12.10 and 12.20, four aromatic protons at δ 6.65 (d, J=3.0 Hz), 7.13 (brs), 7.25 (d, J=3 Hz) and 7.55 (brs) and a methyl at 2.40. These data along with UV and IR data (which are in accord with literature values [36]) explain the structure for emodin.



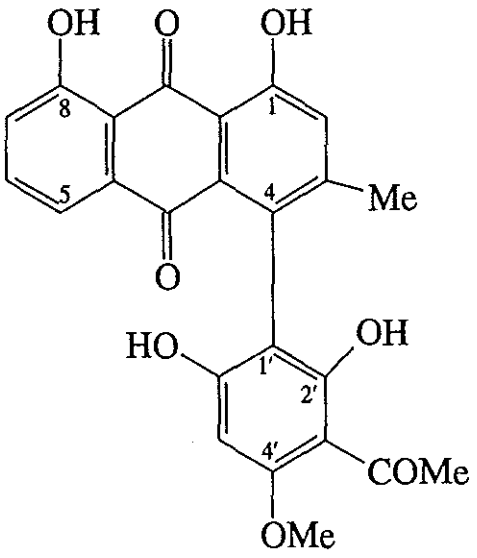
Emodin (125)

Knipholone (126)

This is a reddish pigment which turned deep red upon spraying with 5% KOH in methanol. From its ^{1}H-NMR spectrum, three chelated hydroxyl groups were observed at δ 14.19, 12.59, and 11.98. In addition to this, there are signals for an aromatic methyl at δ 2.15, a methyl on a carbonyl carbon at δ 2.65, a methoxy at δ 3.97 and there aromatic protons at δ 6.14 (s, 5'-H) and δ 7.60 (d, J=8.0 Hz, 2H, 5 & 6-H). There is also an overlapped signal with $CDCl_3$ at δ 7.30. Most of the chemical shifts as well as its UV and IR data very well agree with literature value reported for that of knipholone [90] (see Table 10). Knipholone, though claimed to be a taxonomic marker for *Kniphofia* species [91], is reported for the first time from the genus *Senna*.

Table:10: Comparison of ^{1}H-NMR Spectral data of Knipholone (**126**) (90 MHz, $CDCl_3$) with those reported in the literature (400 MHz, Acetone-d_6) [90]

H	**126** δ (ppm)	Knipholone (literature) δ (ppm)
1-OH	11.98 s	12.00 s
2-H	overlap with $CDCl_3$	7.32 (qu, J=0.7 Hz)
3-Me	2.15 s	2.17 (d, J=0.7 Hz)
5-H	7.60 (d, J=8.0 Hz)	7.75 (dd J=8.0, 1.5 Hz)
6-H	7.60 (d, J=8.0 Hz)	7.56 (dd, J=8.0, 1.5 Hz)
7-H	overlap with $CDCl_3$	7.30 (dd, J=8.0, 1.5 Hz)
8-OH	12.59 s	12.53 s
2'-OH	14.19 s	14.22 s
3'-COMe	2.65 s	2.62 s
4'-OMe	3.97 s	3.98 s
5'-H	6.14 s	6.24 s

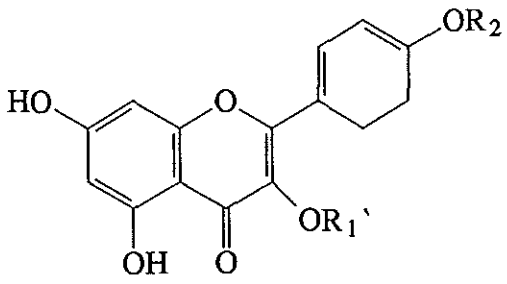


KNIPHOLONE (126)

2.2 THE METHANOL EXTRACT

The methanol extract was concentrated to dryness and poured into cold water to give an aqueous layer and a precipitate. The precipitate was filtered and dissolved in ethyl acetate. The ethyl acetate insoluble part (700 mg) after dissolving in acetone was mixed with 2 g of silica gel dried and transferred on the top of the column which was packed with ethyl acetate and elution was continued with increasing polarity by adding methanol. Three fractions were collected. The first fraction (EtOAc eluate) was repeatedly washed with chloroform and small amount of methanol and dried. The dried material is a yellow powder which fluoresces under UV (366 nm) light and turned deep yellow and brownish when sprayed with 5% methanolic KOH and 0.5% fast blue B-5% methanolic KOH, respectively.

Its ^{1}H-NMR data indicate the presence of chelated hydroxyl group at δ 12.45 two meta coupled protons at δ 6.62 and 6.45, four ortho coupled protons at δ 6.90 and 7.65 and a methoxy at δ 3.83. These data well explain the following two alternative structures (127 & 128).



127 : R_1 = H , R_2 = Me

128 : R_2 = Me , R_2 = H

The absence of a singlet in the ^{1}H-NMR at about δ 6.40-7.20 strongly suggests that the proton at C-3 is substituted. The methoxy is not positioned on C-7. This is because in 5,7-dihdroxy substitution, the ranges of δ are 6.16-6.25 for H-6 and 6.39 -

6.56 for H-8. Methylation or glucosylation of the 7-OH shifts these signals downfield to 6.33-6.48 and 6.71-6.93 ppm respectively [92]. So the methoxy may be either at the C-3 or C-4'.

From its UV spectrum, the absorption maximum of band I being at 369 nm is an indication of a flavonol skeleton having free OH at C-3 [75]. The bathochromic shift (31 nm with respect to the methanol spectrum) with decreasing intensity of band I when NaOH is used as shift reagent also suggests free OH at C-3. This is further confirmed by the bathochromic shift 59 and 56 nm of band I (cf. MeOH) using $AlCl_3$ and $AlCl_3$/HCl shift reagents, respectively [75,85]. The change in absorption maximum of band II in NaOAc spectrum with respect to that of MeOH being 21 nm is quite suggestive of the presence of an OH at C-7. This is also indicated by the appearance of new band (cf. MeOH) at 326 nm in the NaOH spectrum [75].

Its IR spectrum indicates an OH, aryl-H and aryl-carbonyl stretching at 3241, 3075 and 1656 cm^{-1} respectively.

The UV data (see Table 11) in conjunction with the ^{1}H-NMR data very well explain the structure of kaempferol 4'-methyl ether which is, though isolated from many plant species, not known for the genus *Senna* and its ^{1}H-NMR in DMSO-d_6 is reported for the first time.

Table 11. UV absorption maxima (λ_{max} nm, logϵ) of **127** and those of kaempferol-3 and -4'-methyl ethers reported in the literature [93,94]

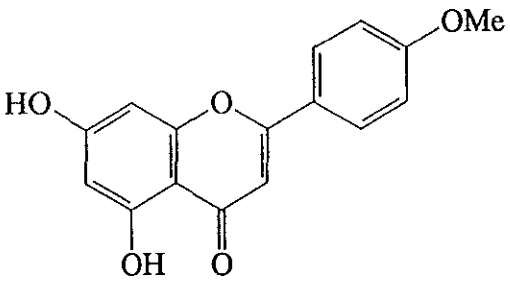
Compounds	Reagents					
	MeOH	$AlCl_3$	$AlCl_3$ +HCl	NaOAc	NaOAc +HCl	NaOH (NaOMe)
	204 (4.5)	241sh	242sh	220 (4.63)	223 (4.62)	215 (4.55)
127	255 (4.29)	266 (4.36)	264 (4.29)	276 (4.28)	269 (4.28)	244 (4.17)
	370 (4.27)	429 (4.33)	361 (3.99)	322 (4.05)	349 (3.94)	275 (4.07)
		360	426 (4.27)	393 (4.24)	427 (4.23)	327 (4.29)
					400 (3.75)	
Kaempferol 4'-OMe	270 (1.0)	235 (0.69)	235 (0.70)	274 (1.2)		(280), (1.2)
	330 (0.60)	245 (0.60)	245 (0.60)	301sh (0.8)		(325sh), (0.50)
	370 (1.0)	270 (0.90)	270 (0.90)	384 (1.0)		(415), (1.0)
		310 (0.30)	310 (0.30)			
		355 (0.40)	350 (0.50)			
		425 (1.0)	425 (1.0)			
Kaempferol 3-OMe	268	277	277	277		(275)
	299sh	305	305	310		(325)
	349	350	347	389		(396)
		399	399			

The ethyl acetate extract of the aqueous layer was concentrated to dryness (1.6 g) and chromatographed (silica gel, $CHCl_3$-EtOAc gradient elution). The first fraction was found to be a mixture of trace anthraquninones while the second fraction was dried and repeatedly washed with chloroform and a few drops of methanol to give a yellow powder (7 mg) which fluoresces under UV light and turned deep yellow upon spraying with 5% methanolic KOH. Its ^{1}H-NMR data revealed that it is a mixture of two flavonoids, one of which is identified to be acacetin (**129**) and the data agreed very well with the literature value (Table 12) [92]. The other component of the mixture is found to be 3,5,6,7,8,3',4'-heptahydroxy flavone (**130**).

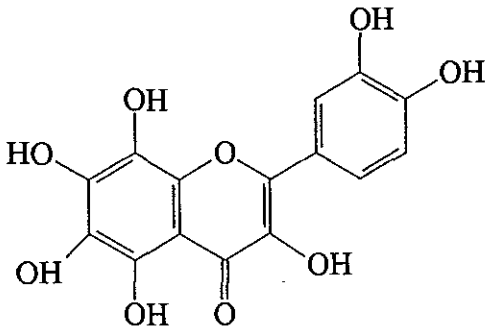
Table 12 : Comparison of ^{1}H-NMR (300 MHz) data of mixture of **129** and **130** with those of acacetin reported in the literature [92]

H	**129** δ/ppm (acetone-d_6)	**130**	Acacetin[83] δ/ppm (DMSO-d_6)
2'	7.96 (d, J=8.9 Hz)	7.55 (d, J=1.94 Hz)	8.03 (d, J=9.0 Hz)
6'	7.96 (d, J=8.9 Hz)	7.50 (dd, J=8.3, 1.94 Hz)	8.03 (d, J=9.0 Hz)
3'	7.02 (d, J=8.9 Hz)	.....	7.11 (d, J=9.0 Hz)
5'	7.02 (d, J=8.9 Hz)	6.85 (d, J=8.3 Hz)	7.11 (d, J=9.0 Hz)
3	6.62s	.....	6.87 (s)
5-OH	13.00 (s)	13.00 (s)	13.00 (s)
6	6.24 (d, J=2.0 Hz)	.....	6.20 (d, J=2.0 Hz)
7-OH	.....	.....	.....
8	6.53 (d, J=2.0 Hz)	.....	6.51 (d, J=2.0 Hz)

Both acacetin (129) and 3,5,6,7,8,3',4'-heptahydroxyflavone are not known for the genus *Senna*.



Acacetin (129)



3,5,6,7,8,3′,4′-Heptahydroxy flavone (130)

3.0 EXPERIMENTAL

Plant material: The pods and seeds of *S. didymobotrya* were collected from Addis Ababa University garden on Nov. 14/ 1994, and identified by Dr. Sebsebe Demissew, The National Herbarium, Biology Department, Addis Ababa University (Voucher No.AH-2 & 3).

3.1 GENERAL:

Instruments:

UV: Milton Roy Sepctronic 1001 Plus

IR: Perkin-Elmer FTIR 1600 Series

^{1}H-NMR 90 (Joel Fx 90Q) and 300 MHz

MP Kofler block hot stage melting point apparatus, and are uncorrected

Chromatography:

Analytical TLC Silica gel (Merk) coated on aluminum foil 0.25 mm thickness

Preparative TLC Silica gel 60 $PF_{254+366}$ (Merck)

Column chromatography Silica gel 60 (0.040-0.063 mesh ASTM) (Merck)

3.2 EXTRACTION AND ISOLATIONS

Seeds: The powdered seeds of *S.didymobotrya* (1 Kg) were soaked in 2.5 l acetic acid (5% in water) for 24 hrs and dried. The dried material was defatted with cold petroleum ether (4.6 l) and the mark was exhaustively extracted with cold chloroform (6 l). The chloroform extract, upon concentrating gave 7.2 g of a brown residue. This residue showed no characteristic coloured spots on TLC upon spraying with 5% methanolic KOH.

Pods: The dried and ground pods (1.5 kg) were soaked in 5% acetic acid (5 l) for 24 hrs and dried. The acid treated material was then defatted with cold pet. ether (40-60°C) (30 l) and the marc was extracted with chloroform (30 l) and methanol (22 l) successively. The chloroform extract, upon evaporating of the solvent, gave a dark residue (21.4 g, 1.4%) which on TLC (solvent system $CHCl_3$) showed 4 coloured spots with R_f values of 0.57, 0.43, 0.34 and 0.17. The methanol extract gave brownish gummy like residue (170 g, 11.3%).

3.3 CHLOROFORM EXTRACT

Silica gel (300 g) impregnated with 0.5 N oxalic acid was packed with chloroform into a glass column. The dark residue (10 g) was mixed with 30 g of silica gel (oxalic acid washed) and after drying transferred to the top of the column and elution with chloroform was continued. Seven fractions were collected. The first fraction gave chrysophanol (**120**) and physcion (**121**), fractions two to six were combined based on their TLC similarity and separated on PTLC (Silica gel, $CHCl_3$) to yield **122** and **124**. The seventh fraction was fractionated on Sephadex (LH-20, $CHCl_3$:MeOH 2:1) into chlorophyll and two coloured fraction. The second coloured fraction was separated on PTLC eluting with pet.ether: EtOAc (4:1) to yield emodin (**125**) and knipholone (**126**).

Chrysophanol (**120**) . ^{1}H-NMR (90 MHz, $CDCl_3$): δ 12.00 (s, OH), 12.10 (s, OH), 7.65 (brs, 1H, 4-H), 7.80 (1H, 5-H), 7.58 (1H, 6-H), 7.32 (1H, 7-H), 7.10 (brs, 1H, 2-H), 2.43 (s, 3H, 3-Me); UV λ^{MeOH}_{max} nm (logϵ): 225 (4.13), 254 (3.90), 278, 289, 429 (3.61); IR ν^{KBr}_{max} cm^{-1} : 3431, 1730, 1673, 1628.

Physcion (**121**). ^{1}H-NMR (90 MHz, $CDCl_3$): 12.10 (s, OH), 12.30 (s, OH), 7.60 (brs, 1H, 4-H), 7.35 (d, 1H, 5-H, J=3 Hz), 7.05 (brs, 1H, 2-H), 6.50 (d, 1H,7-H J=3 Hz), 3.90 (s, 3H, 6-OMe), 2.40 (s, 3H, 3-Me); UV λ^{MeOH}_{max} nm (logϵ): 252 (4.44), 265 (4.43), 286 (4.40), 434 (4.20); IR ν^{KBr}_{max} cm^{-1}: 3427, 1675, 1629.

10-(Physcion-7'-yl)-10-hydroxy chrysophanol anthrone (**122**). ^{1}H-NMR: see Table 9 MS m/z (rel. int. %): 538.20 $[M]^+$ (48), 520.20 $[M-H_2O]^+$ (100), 284.20 [M-chrysophanol]$^+$ (16), 255.20 [M+H-physcion]$^+$ (40); UV λ^{MeOH}_{max} nm (logϵ): 225 (3.90), 271 (4.10), 303 (3.90), 384 (3.70), 468 (3.50); IR ν^{KBr}_{max} cm-^{1}: 3428, 1667, 1616; mp 182-184°C.

9-(Physcion-7'-yl) 5,10-dihydroxy-2-methyl-1,4-anthraquinone (**124**): mp 150°C (dec.); ^{1}H-NMR (300 MHz, $CDCl_3$): 17.10 (s, OH), 12.25 (s, OH), 12.05 (s, OH), 10.60 (s,OH), 6.95 (s, 1H, 3-H), 6.98 (d, 1H, 8-H, J=8.0 Hz), 7.50 (t, 1H, 7-H, J=8.0 Hz), 7.12 (d, 1H, 6-H, J=8.0 Hz), 7.15 (brs, 1H, 2'-H), 7.70 (brs, 1H, 4'-H), 7.62 (s, 1H, 6'-H), 3.84 (s, 3H, 6'-OMe), 2.45 (s, 3H, 3'-Me), 2.10 (s, 3H, 2-Me); MS m/z (rel. int. %): 536.30 $[M]^+$ (2); UV λ^{CHCl3}_{max} nm (logϵ): 580 (2.75), 536 (3.01), 500 (3.03), 446 (3.64), 300 (3.66), 288 (3.72), 239 (3.95); IR ν^{KBr}_{max} cm^{-1}: 3452, 1731, 1626.

emodin (**125**). ^{1}H-NMR (90 MHz, $CDCl_3$): δ 12.20 (s, OH), 12.05 (s, OH), 7.55 (brs, 1H, 4-H), 7.25 (d, 1H, 5-H, J=3.0 Hz), 7.13 (brs, 1H, 2-H) and 6.65 (d, 1H, 7-H,

J=3.0 Hz), 2.45 (s, 3H, 3-Me); UV λ^{MeOH}_{max} nm (logϵ): 253 (4.31), 265 (4.30), 289 (4.33) 436 (4.03); IR ν^{KBr}_{max} cm^{-1}: 3394, 1701, 1629.

knipholone (**126**). UV λ^{MeOH}_{max} nm (logϵ): 226 (4.96), 255 (4.86), 288 (4.84), 429 (4.45); IR ν^{KBr}_{max} cm^{-1}, 3538 (OH), 2920, 1706, 1603, 1457, 1361, 1274; ^{1}H-NMR: see Table 10.

3.4 METHANOL EXTRACT

The concentrated methanolic extract was poured into cold water from which an aqueous solution (fraction 1) and coloured residue (fraction 2) were obtained.

Fraction 1 was extracted with ethyl acetate and concentrated to dryness (1.6 g) . The concentrate was chromatographed on a silica gel column and eluted with chloroform-ethyl acetate (gradient elution). The first fraction gave a mixture of trace anthraquinones. The second eluate was dried and repeatedly washed with chloroform and a few drops of methanol to give a yellow powder which was found to contain mixture of two flavonoids, acacetin (**129**) and 3,5,6,7,8,3',4'-heptahydroxyflavone (**130**).

Fraction 2 was dissolved in ethyl acetate. The ethyl acetate insoluble part was dissolved in acetone and filtered. The filtrate was concentrated to dryness and chromatographed (Silica gel, EtOAc, EtOAc:methanol 4:1) to give three fractions, F_1, F_2 and F_3. F_1 was repeatedly washed with chloroform and small amount of methanol to give kaempferol 4'-methyl ether (**127**).

kaempferol 4'-methyl ether (**127**). ^{1}H-NMR (DMSO-d_6): δ 12.45 (s, 5-OH), 7.65 (d, 2H, 6'-H & 2'-H ,J=8.0 Hz), 6.90 (d, 2H, 5'-H & 3-'H, J=8.0 Hz), 6.45 (d, 1H, 8-H, J=2.0 Hz), 6.15 (d, 1H, 6-H, J=2.0 Hz), 3.85 (s, 3H, 4'-OMe); UV: see Table 11. IR ν^{KBr}_{max} cm^{-1}: 3241, 3075, 1656, 1614.

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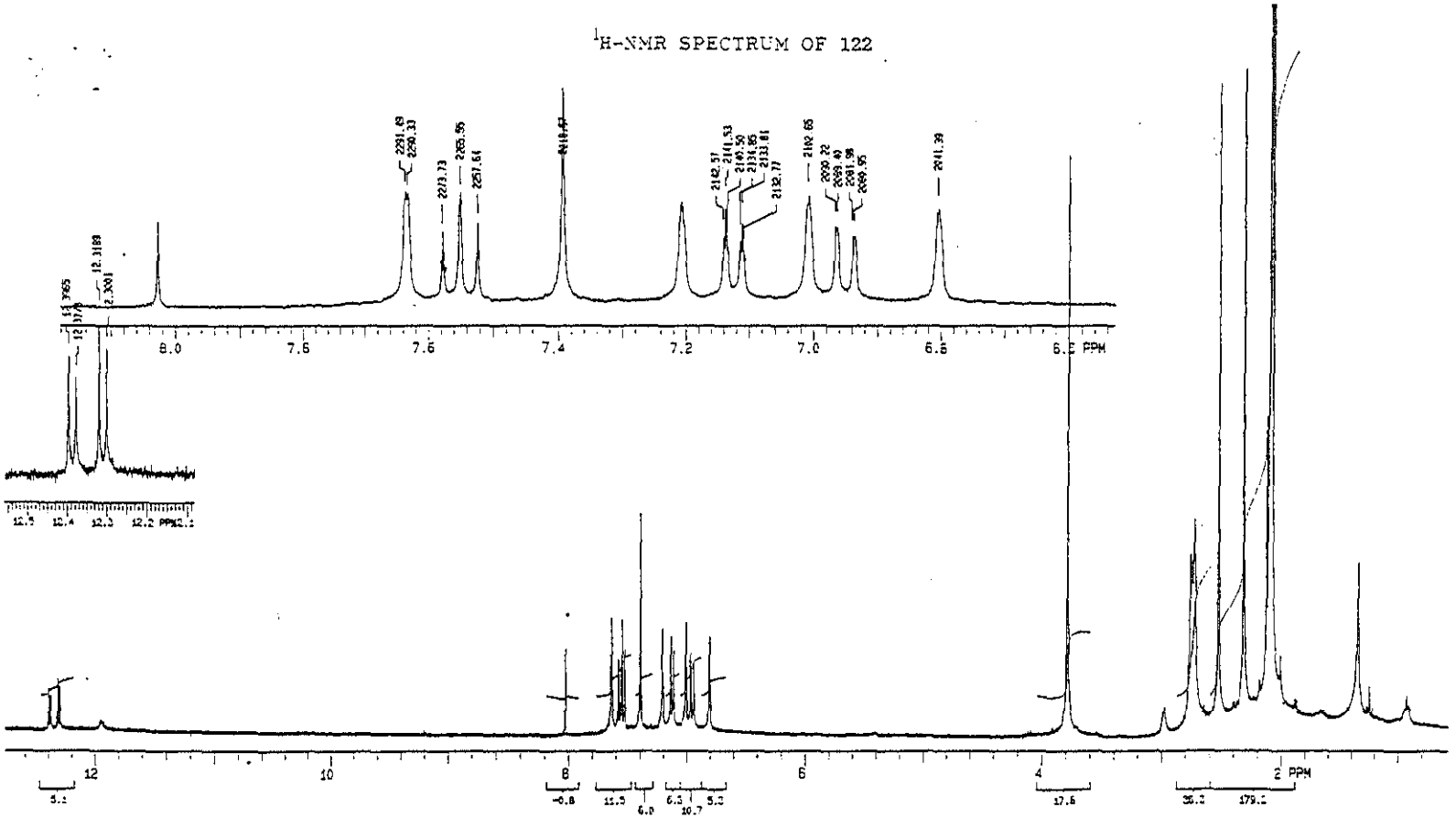
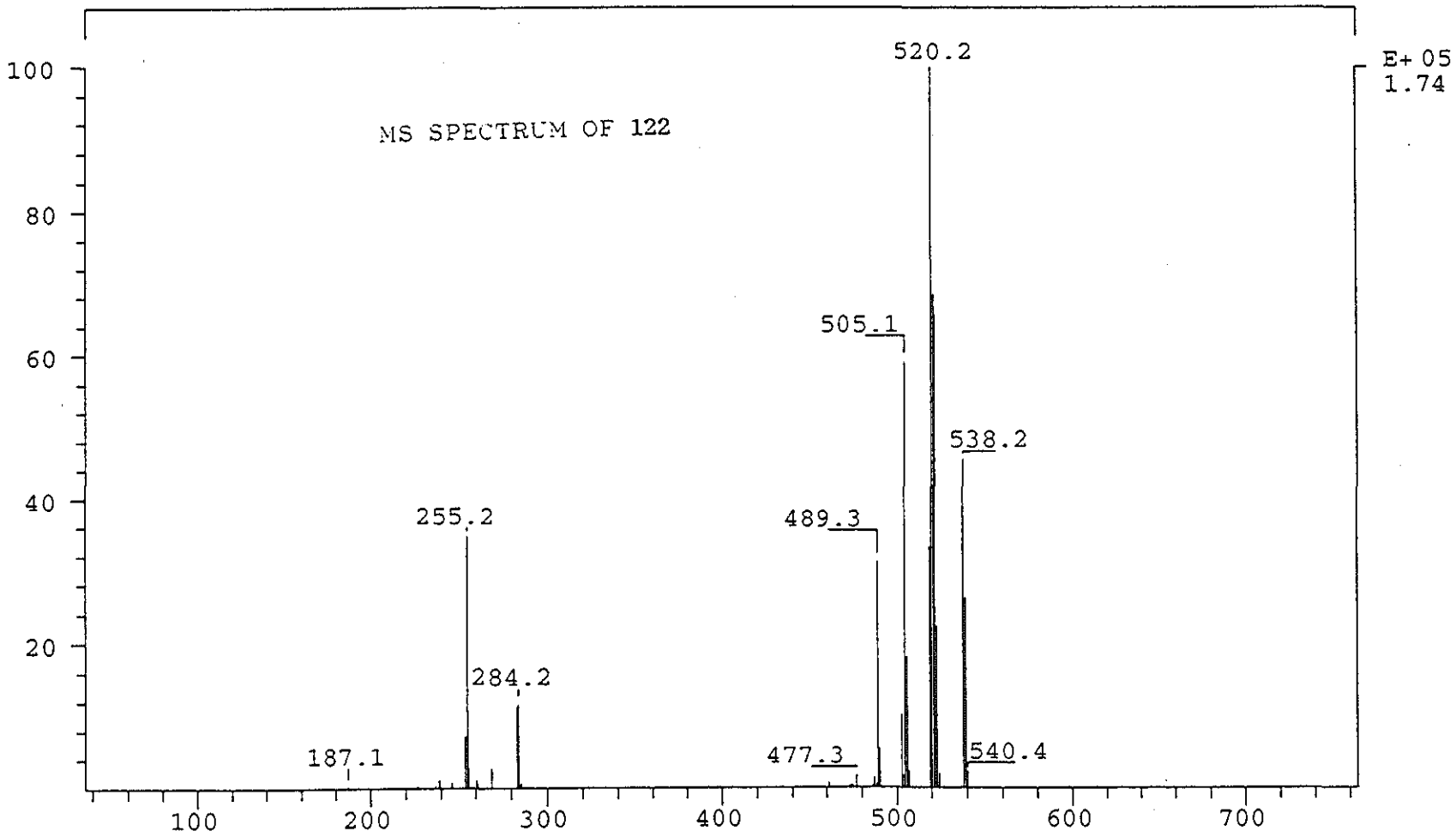
APPENDIX

^{1}H-NMR SPECTRUM OF 122




MS SPECTRUM OF 122
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60
40
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E+ 05
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505.1
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489.3
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540.4
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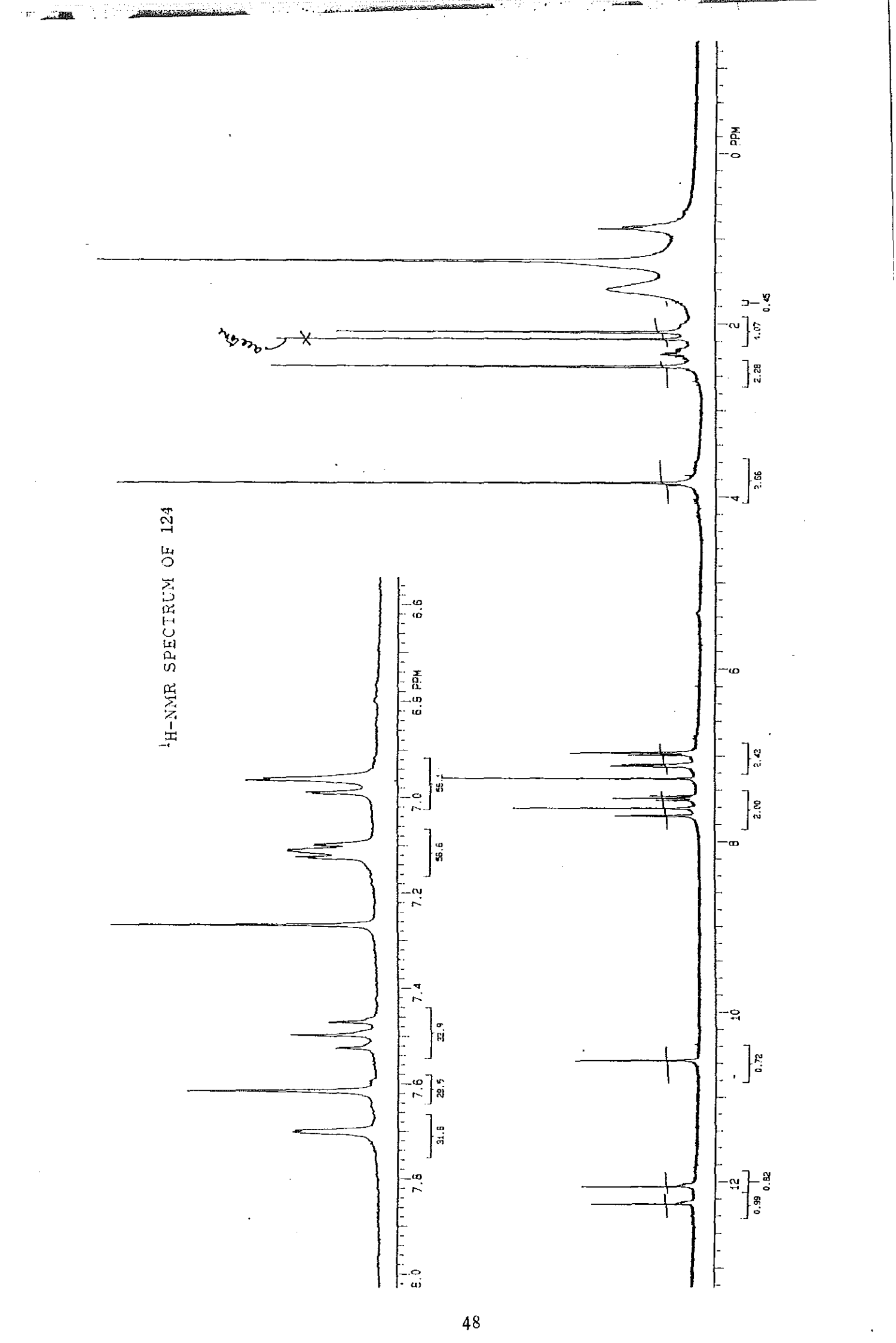
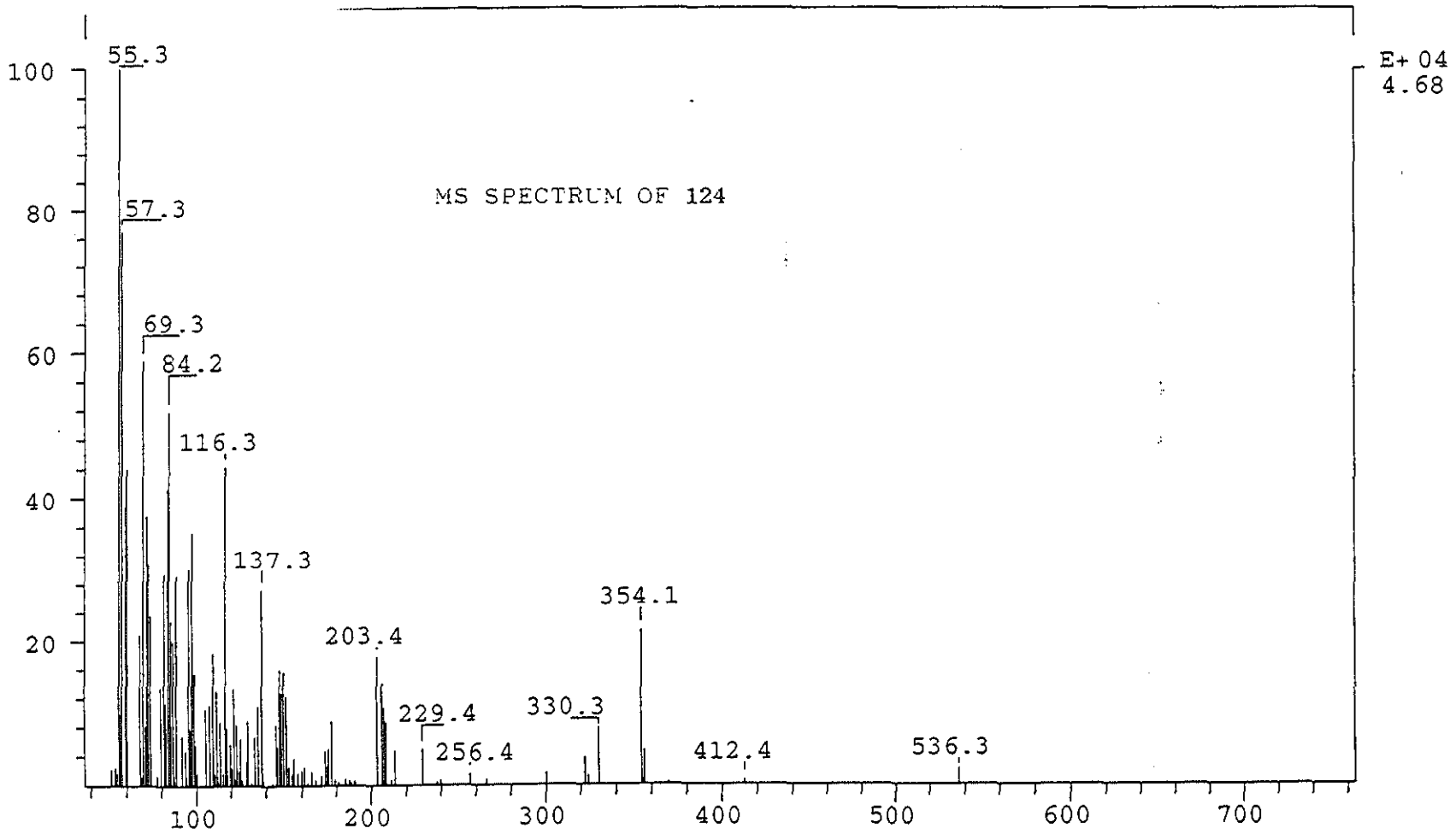
400
500
600
700


^{1}H-NMR SPECTRUM OF 124


MS SPECTRUM OF 124
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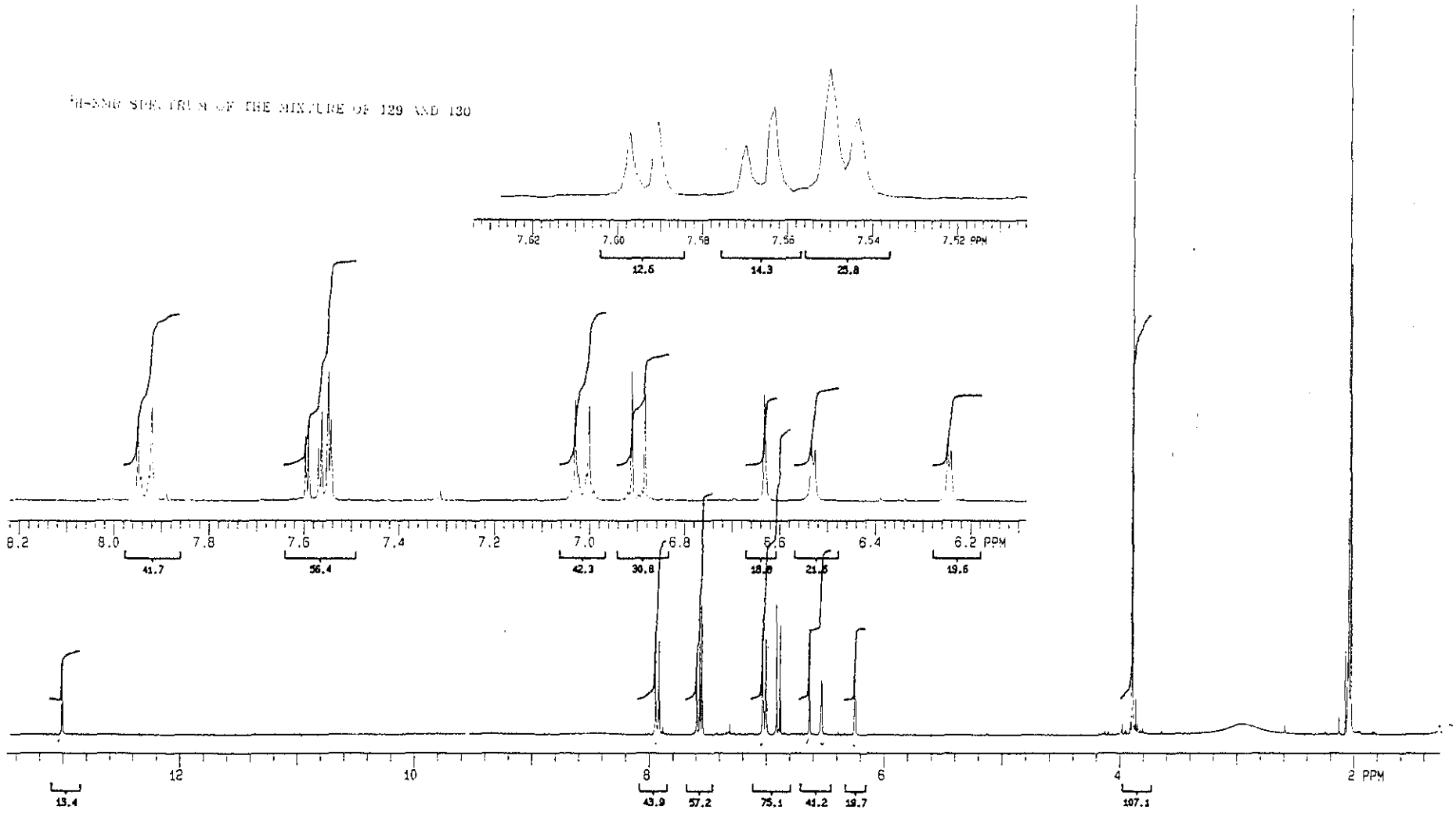
69.3
84.2
116.3
137.3
203.4
229.4
256.4
330.3
354.1
412.4
536.3
100
80
60
40
20
100
200
300
400
500
600
700

^{1}H-NMR SPECTRUM OF THE MIXTURE OF 129 AND 130



^{1}H-NMR SPECTRUM OF THE MIXTURE OF 129 AND 130 (6-8 ppm)