PHYTOCHEMICAL INVESTIGATIONS OF THE STEM BARK OF *SENNA FLORIBUNDA* AND *SENNA DIDYMOBOTRYA*

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Dedicated to

1. Aynalem Adugna who graduated from Bonga TTI on 11th Hamle, 1991 E.C.
2. My families: Ato Adane Bahiru, W/ro Adebut Waktola, Ato Messele Bahiru and W/ro Birnesh Denibel.

ACKNOWLEDGMENTS

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PHYTOCHEMICAL INVESTIGATIONS OF THE STEM BARK OF *SENNA FLORIBUNDA* AND *SENNA DIDYMOBOTRYA*

By

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Advisor: Dr Gizachew Alemayehu

Anthraquinones and a naphthoquinone have been isolated and characterized from the stem barks of *S. floribunda* and *S. didymobotrya.*

The CH_2Cl_2:MeOH (1:1) extract of the stem bark of *S. floribunda* yielded four bianthraquinones: floribundone-1(**43**), 5,7'-physcion-fallacinol (**53**), 5,7'-physcion-physcion anthrone (**83**), 5,5'-physcion-physcion anthrone (**84**) along with three common anthraquinones: physcion, chrysophanol and emodin. The isolation of 5,7'-Physcion-physcion anthrone and 5,5'-physcion-physcion anthrone is reported here for the first time.

The CH_2Cl_2:MeOH (1:1) extract of *S. didymobotrya* bark yielded four compounds: 2-methoxystypandrone (**82**), 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (**70**), fallacinol (**6**) and 10,10'-bichrysophanol (**59**) along with the three common anthraquinones: physcion, chrysophanol and emodin.

The elucidation of the structures was based on spectroscopic data and in some cases by direct TLC comparison with authentic samples.

1.0 INTRODUCTION

1.1 GENERAL

Natural products are naturally occurring organic chemicals that are unique to one organism, or common to a small number of closely related organisms [1,2].

Natural products became a necessity to mankind since antiquity. In ancient times they have been immensely utilized for various purposes, for instance, as foodstuff, weapons, for treatment of diseases, for colouring matters, etc., in their crude form. But when chemists in the late eighteenth century took the final jump from the world of myth into modern science, the true properties of extracts from nature (living organisms) aroused a great curiosity for these chemists. So they began to isolate and finally analyze these extracts produced in the living cells [2]. The isolation methods involve chromatographic techniques: CC, GC, TLC, HPLC and PC. These methods have made it possible to isolate compounds present in extremely small quantity. After obtaining these natural products in pure form, the challenging problem is structural elucidation of the isolated compounds. Two techniques have been developed to solve such a problem. These are

1. Degradation technique

This is technique involves conversion of the isolated compounds to smaller fragments of known structure.

2. Spectroscopic techniques (UV, IR, NMR, MS, CD, ORD, ESR)

These are techniques that enable chemists to elucidate structures with much less sample

and time than were needed earlier by degradation technique.

Natural products play a fundamental role in the coexistence of the species (organisms) in the ecosystem, thereby ensuring their survival [3]. Some of the interactions of the living organisms in the ecosystem are given below, as example.

1. Plant-animal interactions
   - Colour and fragrance of flowering plants attract pollinators from a distance. This role is played by secondary metabolites produced by these plants.
   - Some plants are able to synthesize secondary metabolites that repel or attract herbivores.
2. Animal-animal interactions.
   - Some animals produce toxins for chemical defense and warfare.
   - Other groups of animals can produce secondary metabolites for the purpose of alarming the members of the group from danger, and sex attraction, etc.

There are so many reasons that led organic chemists to the phytochemical investigations of plants. Some of them may be due to the plant's toxicity to human being or animal life, its medicinal value, its physiological activity of various kinds, its possession of unusual pigments or exudates, and so on [2]. The contribution of natural products to the development of medicine could be demonstrated by the amount of plant-derived drugs being used. In general about 40% of modern drugs used today are said to be of natural origin [4].

1.2 *SENNA* SPECIES AND THEIR MEDICINAL USES

The genus *Senna*, in the family of Leguminosae, is known to have about 240 species distributed throughout the tropics and subtopics [5,6]. Several of these species have important medicinal values, and are used both in traditional and modern medicines [7,8]. Majority of people living in the villages of Africa, Asia and other developing countries use traditional medicines, most of which are derived from plants, in order to alleviate their sufferings and for the continued maintenance of their health. Nowadays even people living in developed countries use home-remedies of plant origin. In this context, several *Senna* species have been widely used since ancient times. They are used in the treatment of sexually transmitted diseases, skin disease, cattle disease, malaria, and are sources of well-known senna purgatives. Many of them also found to possess insecticidal and antibiotic properties [4]

The leaves and root parts of *S. occcidentalis* are useful for snake-bites, as antimalarial, as painkiller and for anthelmintic purposes. The dried leaflets of *S. acutifolia* and *S. angustifolia* are well-known purgatives. The aerial parts of *S. obtisfolia* are used as remedy for stomach trouble, for quickening of delivery and as an antibiotic [4].

The pharmacologically active constituents isolated from different parts of these medicinally useful *Senna* species are also widely used for preparation of modern medicines: e.g., Anthraquinone glycosides are important cathartic compounds.

They are used as purgatives and are employed in geriatric and pediatric medicines for their unique pharmacological effects [8].

Flavonoid aglycones and some flavonoids such as quercetin, rhamantin and kaempferol are also pharmacologically active constituents isolated from the *Senna* species, and are used as mutagenic drugs [4].

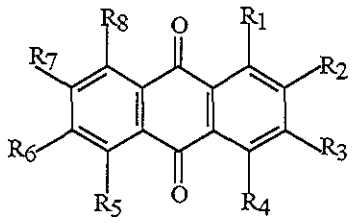
Nowadays the documentation of medicinal uses of these species is becoming increasingly urgent because some of these species are in rapid loss from their natural habitat due to anthropogenic activities. There is also an urgent need to document not only the uses, but also the constituents and their pharmacological activities [9].

1.3 ANTHRAQUINONES AND THEIR DERIVATIVES FROM *SENNA* SPECIES GROWING IN ETHIOPIA

The *Senna* species are rich sources of anthraquinones, preanthraquinones, dimeric anthraquinones, flavonoids, and glycosides of anthraquinones and flavonoids [7]. The medicinal values, colour reactions, biosynthetheses, spectral properties and biological activities of these compounds are well documented [4,10-16].

Anthraquinones, preanthraquinones and bianthraquinones isolated so far from Ethiopian *Senna* species are given compiled in Tables 1, 2 and 3, respectively.

Table 1. Anthraquinones isolated from Ethiopian *Senna* species.



Compound 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	[16, 17]
2	Physcion	OH	H	Me	H	H	OMe	H	OH	any *Senna* species	[16,17]
3	Emodin	OH	H	Me	H	H	OH	H	OH	any *Senna* species	[16, 17]
4	Aloe-emodin	OH	H	CH_2OH	H	H	H	H	OH	*S. didymobotrya* *S. obtusfolia*	[18,19, 20]
5	Rhein	OH	H	CO_2H	H	H	H	H	OH	*S. didymobotrya* *S. alexandrina* *S. siamea*	[18, 19, 21, 22]

Table 1. Continued.....

Compound No	Name of the compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Source	References
7	Islandicin	OH	H	Me	OH	H	H	H	OH	*S. occidentalis*	[17, 23]
8	Helminthosporin	OH	H	Me	H	OH	H	H	OH	*S. occidentalis*	[23]
9	Xanthorin	OH	H	Me	H	OH	OMe	H	OH	*S. sophera* *S. occidentalis*	[16, 23, 24]
10	1,4,5-Trihydroxy-7-methoxy-3-methyl anthraquinone	OH	H	Me	OH	OH	H	OMe	H	*S. occidentalis*	[17, 25]
11	Sopheranin	OH	Me	OH	H	H	OH	$CH=CH_2$	OH	*S. sophera*	[26]
12	1,2,7-Trihydroxy-6,8-dimethoxy-3-methyl anthraquinone	OH	OH	Me	H	H	OMe	OH	OMe	*S. sophera*	[26]
13	1,2,6-Trihdroxy-7,8-dimethoxy-3-methyl anthraquinone	OH	OH	Me	H	H	OH	OMe	OMe	*S. sophera*	[26]

Table 1. Continued.....

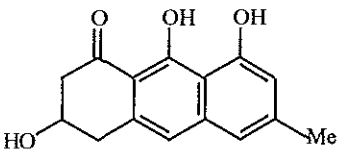
Compound No	Name of the compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Source	References
14	1,3-Dihydroxy-5,7,8-trimethoxy-2-methyl anthraquinone	OH	Me	OH	H	OMe	H	OMe	OMe	*S. sophera*	[16, 17]
15	1,8-Dihydroxy-3,6-dimethoxy-2-methyl-7-vinyl anthraquinone	OH	Me	OMe	H	H	OMe	CH_2=CH	OH	*S. sophera*	[16, 27]
16	2,7-Dihydroxy emodin-6,8-dimethyl ether	OH	OH	Me	H	H	OMe	OH	OMe	*S. sophera*	[16]
17	2,7-Dihydroxy emodin-7,8-dimethyl ether	OH	OH	Me	H	H	OH	OMe	OMe	*S. sophera*	[16]
18	1,4,8-Trihydroxy-6-methoxy-2-methyl ether	OH	Me	H	OH	H	OMe	H	OH	*S. occidentalis*	[17]
19	Xanthorin-5-methyl ether	OH	H	Me	H	OMe	OMe	H	OH	*S. sophera* *S. occidentalis*	[17]

Table 1. Continued.....

Compound No	Name of the compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Source	References
20	Obtusin	OMe	OH	Me	H	H	OMe	OMe	OH	*S. obtusfolia*	[16, 20, 28]
21	Auranthio-obtusin	OMe	OH	Me	H	H	OH	OMe	OH	*S. obtusfolia*	[16, 20, 28]
22	Obtusfolin	OMe	OH	Me	H	H	H	H	OH	*S. obtusfolia*	[16, 20]
23	Chryso-obtusin	OMe	OH	Me	H	H	OMe	OMe	OMe	*S. obtusfolia*	[16, 20, 28]
24	Questin	OH	H	Me	H	H	OH	H	OMe	*S. obtusfolia*	[16, 29]
25	1-De-O-methyl chryso-obtusin	OH	OH	Me	H	H	OMe	OMe	OMe	*S. obtusfolia*	[16]
26	1-De-O-methyl obtusin	OH	OH	Me	H	H	OMe	OMe	OH	*S. obtusfolia*	[16]
27	1-De-O-methyl auranthio-obtusin	OH	OH	Me	H	H	OH	OMe	OH	*S. obtusfolia*	[16]
28	Chrysophanol-8-methyl ether	OH	H	Me	H	H	H	H	OMe	*S. obtusfolia*	[17]

Table 1. Continued.....

Compound No	Name of the compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Source	References
29	1,4,5-Trihydroxy-7-methyl anthraquinone	OH	H	H	OH	OH	H	Me	OH	*S. occidentalis*	[17, 25]
30	1,8-Dihydroxy-2-methyl anthraquinone	OH	Me	H	H	H	H	H	OH	*S. occidentalis*	[17, 25]
31	Parietenic acid	OH	H	CO_2H	H	H	OMe	H	OH	*S. didymobotrya*	[18]
32	Nataloe-emodin	OH	H	Me	H	H	H	OH	OH	*S. longiracemosa*	[30]
33	Isophyscion	OH	H	Me	H	H	H	OMe	OH	*S. longiracemosa*	[30]
34	7-Methyl physcion	OH	H	Me	H	H	OMe	Me	OH	*S. longiracemosa*	[31, 32]



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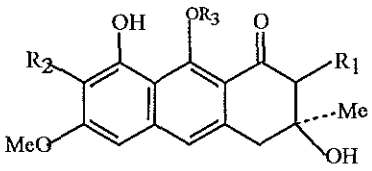
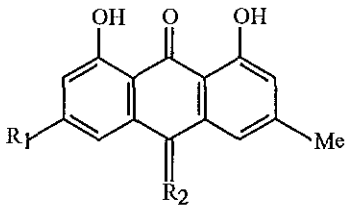


	R_1	R_2	R_3
36.	OH	Me	H
37.	OH	Me	Me
38.	H	H	H
39.	H	Me	H

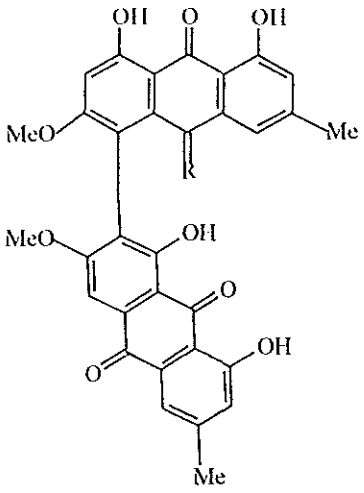


40. R_1 = MeO, R_2 = O

41. R_1 = H, R_2 = H_2

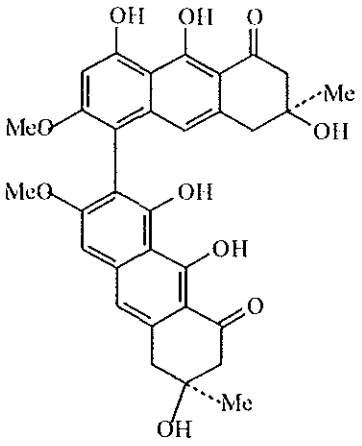
Table 2. Preanthraquinones from Ethiopian *Senna* species

Compound No	Name of the compound	Source	References
35	Germichrysone	*S. sophera* *S. singueana*	[17, 32, 33, 34, 35, 36, 54]
36	Germitorosone	*S. sophera*	[33, 34, 35, 36, 54]
37	Methyl germitorosone	*S. sophera*	[33, 34, 35, 36, 54]
38	Torosachrysone	*S. sophera* *S. didymobotrya* *S. floribunda* *S. obtusfolia* *S. multiglandulosa* *S. singueana*	[4, 16, 17, 18, 28, 29, 32, 33, 35, 38, 39, 50, 54]
39	7-Methyl torosachrysone	*S. sophera*	[36]
40	Physcion-9-anthrone	*S. sophera*	[35]
41	Chrysophanol-9-anthrone	*S. siamea* *S. alexandrina*	[21, 22]



42. R = H_2

43. R = O



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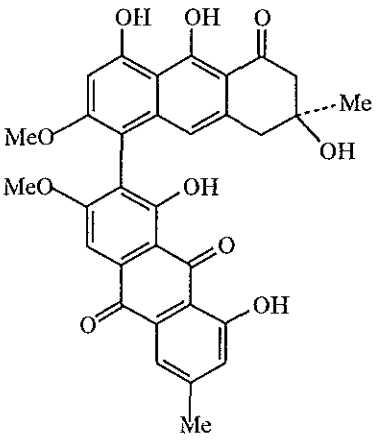

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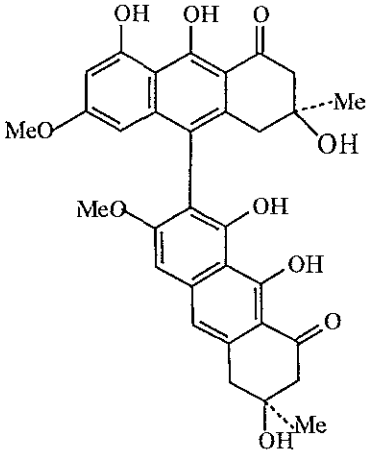

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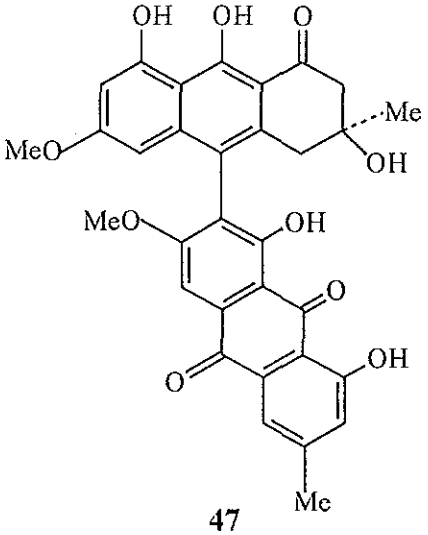

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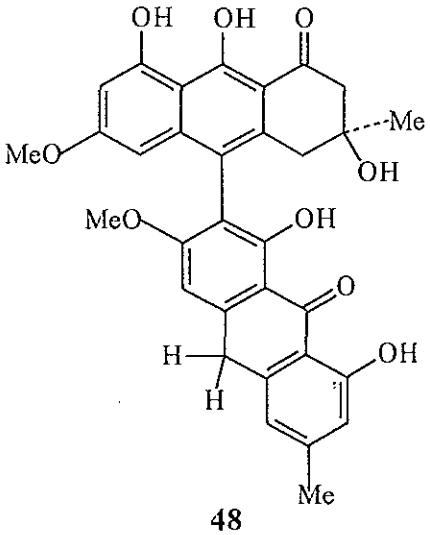

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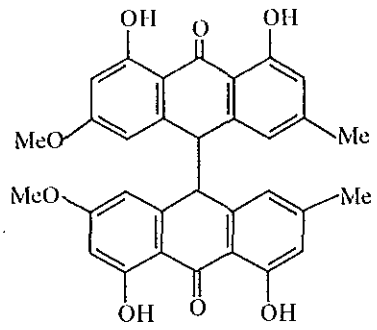

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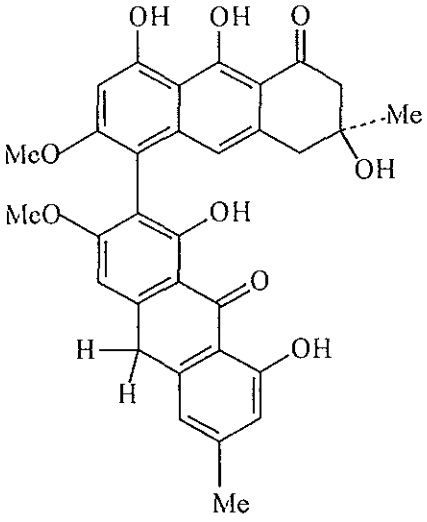

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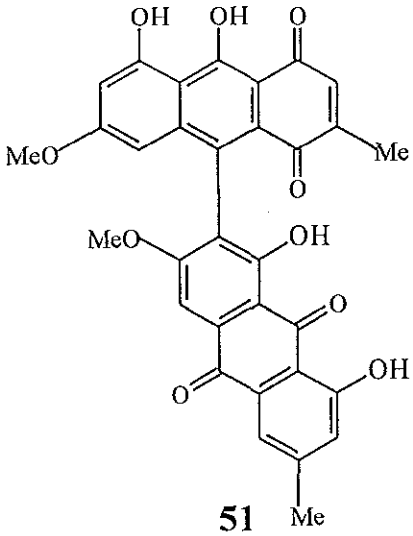

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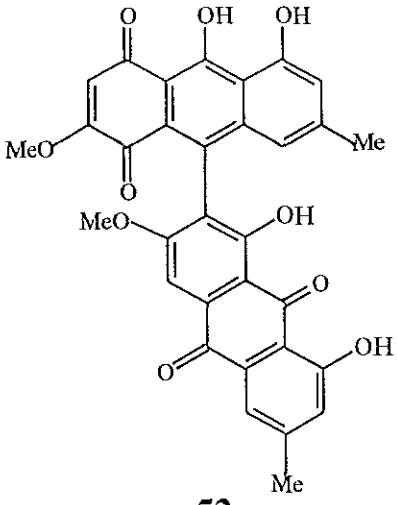

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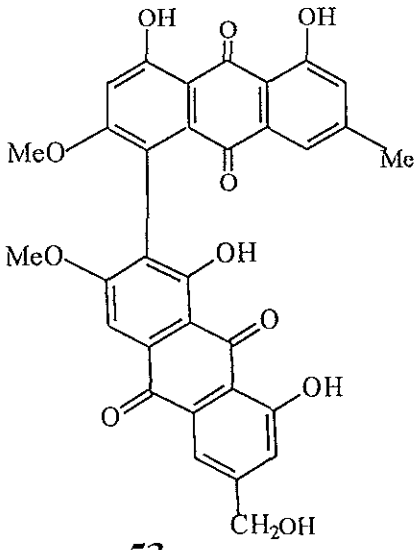

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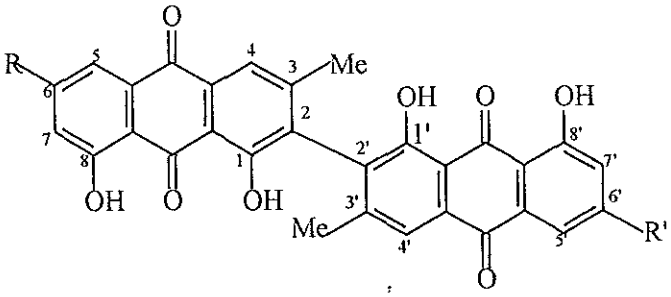
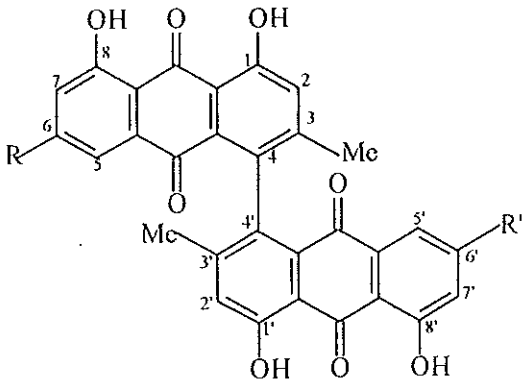


54. R = H, R' = OH

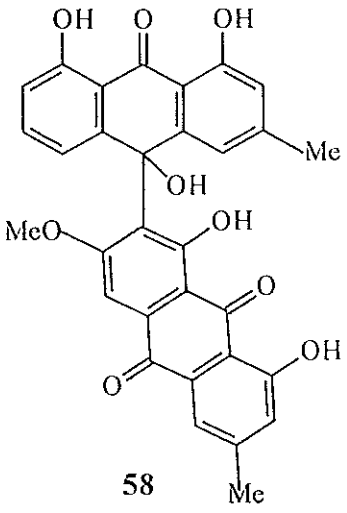
55. R = R' = OH

56. R = R' = H



57. R = R' = H

61. R = H, R' = OH



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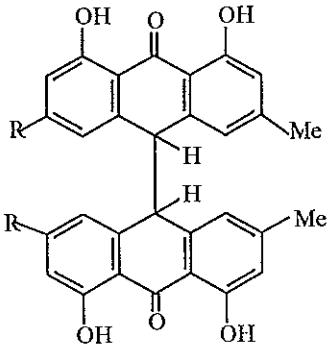
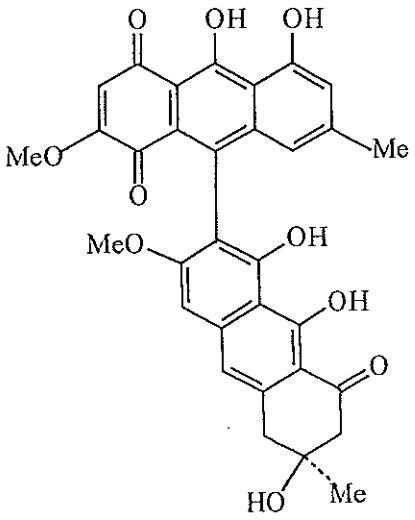


59. R = H

62. R = MeO



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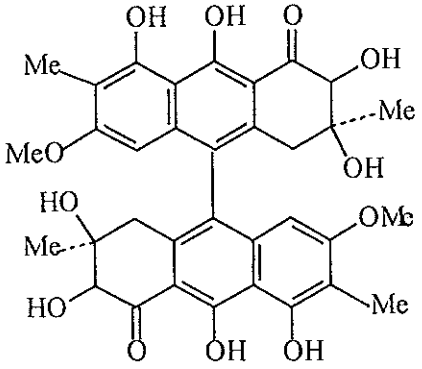

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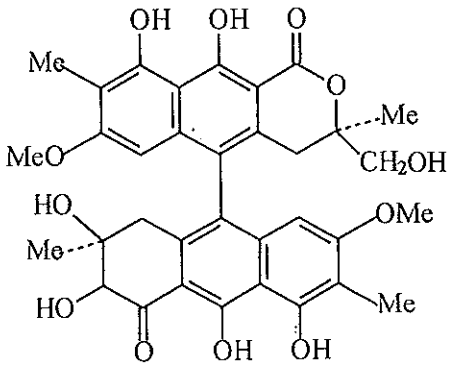

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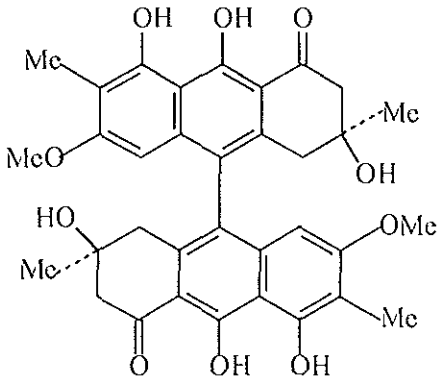

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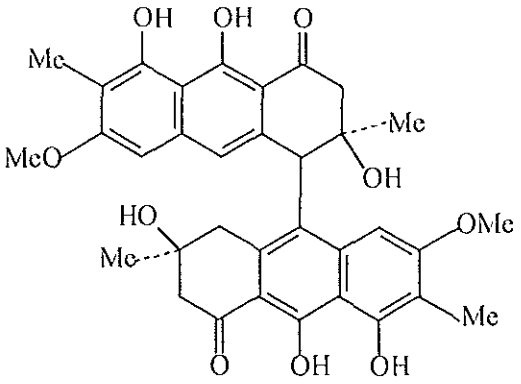

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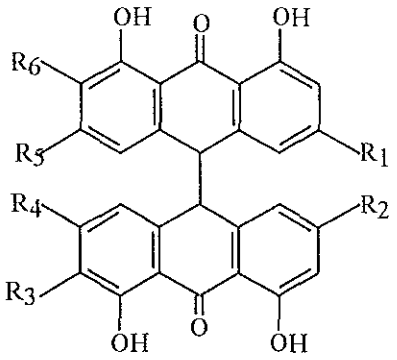

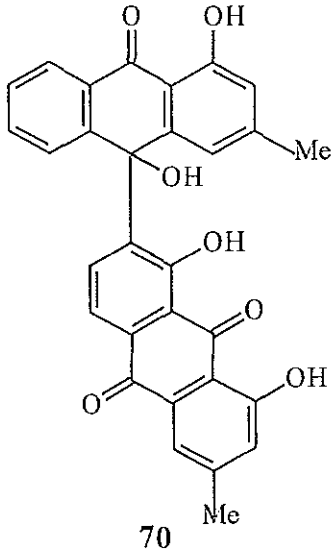

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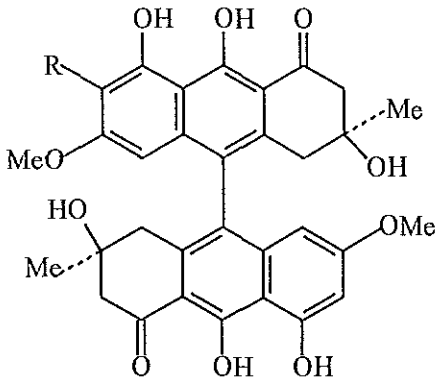
	R_1	R_2	R_3	R_4	R_5	R_6
67	CH_2OH	Me	H	OH	H	H
68	CH_2OH	CH_2OH	H	H	H	H
69	Me	Me	OMe	H	H	H
71	CO_2H	Me	H	H	H	H
72	Me	Me	H	H	OH	H
73	CH_2OH	Me	H	H	H	H
74	Me	Me	H	OH	OH	H
78	CO_2H	Me	H	OH	H	H
79	CO_2H	CO_2H	H	H	H	H
80	CO_2H	CH_2OH	H	H	H	H
81	Me	Me	OMe	H	H	OMe



75. R = Me
76. R = H

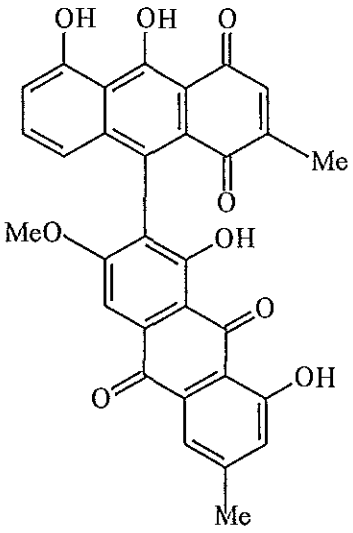


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Table 3. Bianthraquinones isolated from Ethiopian *Senna* species

Compound No	Name of the compound	Source	References
42	Floribundone-1	*S. floribunda*	[38, 46]
43	Floribundone-2	*S. sophera* *S. multiglandulosa* *S. floribunda*	[4, 13, 24, 38, 42, 46, 49, 50]
44	Torosaol-III	*S. sophera*	[38]
45	Torosanin-9,10-quinone (7,5'-Physcion-torosachrysone)	*S. sophera* *S. floribunda* *S. multiglandulosa*	[4, 13, 38, 46]
46	Phlegmacine A_2 and B_2	*S. sophera*	[33, 35, 36]

Table 3. Continued...

Compound No	Name of the compound	Source	References
60	Presengulone	*S. sophera*	[24]
61	Siameadin (4,4'-chrysophanol-emodin bianthrone	*S. occidentalis*	[17]
62	Palmidin D (10,10'-chrysopha nol - physcion bianthrone	*S. longiracemosa* *S. occidentalis* *S. alexandrina*	[17, 21, 30]
63	Torosaol-I	*S. sophera*	[36]
64	Torosaol-II	*S. sophera*	[36]
65	Singuealone-I	*S. sophera* *S. singueana*	[32, 36]
66	Singuealone-II	*S. singueana*	[32]
67	Palmidin A (10,10'-aleo-emodin-emodin bianthrone	*S. alexandrina*	[21]
68	Aloe-emodin-10,10'-bianthrone	*S. alexandrina*	[21]
69	10,10'-Chrysophanol-isophyscion bianthrone	*S. longiracemosa*	[30]
70	10-(Chrysophanol-7'-yl)-10-hydroxychrysophanol	*S. longiracemosa*	[30]
71	Rheidin B (10,10'-rhein-chrysophanol bianthrone	*S. alexandrina*	[21]
72	Palmidin C (10,10'-emodin-chrysophanol bianthrone	*S. alexandrina*	[21]

Table 3. Continued...

Compound No	Name of the compound	Source	References
73	Palmidin B (aloe-emodin-chrysophanol bianthrone)	*S. alexandrina*	[21]
74	10,10'-Emodin bianthrone	*S. alexandrina*	[21]
75	Occidentalol-I	*S. occidentalis*	[56]
76	Occidentalol-II	*S. occidentalis*	[56]
77	5,10-Dihydroxy-2-methyl-9-(physcion-7'-yl)-1,4-anthraquinone	*S. didymobotrya*	[12, 47]
78	Rheidin A (rhein-emodin bianthrone)	*S. alexandrina*	[21]
79	Rhein-10,10'-bianthrone	*S. alexandrina*	[21]
80	Rhein-aloe-emodin-10,10'-bianthrone	*S. alexandrina*	[21]
81	10,10'-Biisophyscion	*S. longiracemosa*	[30]

1.4 CHEMOTAXONOMIC SIGNIFICANCE OF ANTHRAQUINONES AND THEIR DERIVATIVES ISOLATED FROM *SENNA* SPECIES

Natural products are useful in taxonomic studies at the species or generic level. This is because secondary metabolites are unique, some are found only in a single plant species, and many are characteristic of restricted groups of plants. The chemotaxonomic utility of secondary metabolites is that if structurally similar compounds, derived from different plant species, are shown to share the same biosynthetic pathway, both species are tentatively assigned to the same genus or family [3]. Of course many plants are already assigned to particular genera or families based on morphological variations, but biogenetic relationships are helpful in providing confirmations of the accuracy of these assignments. So in some instances chemotaxonomy demonstrates the need for reassignment.

Some of the constituents of *Senna* species, e.g., anthraquinones and their derivatives, are used in chemotaxonomic classification. In order to see the chemotaxonomic significance of anthraquinones and their derivatives, attempt has been made to decide or revise the placement of *C. torosa* by comparing and contrasting the compounds isolated from its different parts with those of isolated from ten Ethiopian *Senna* species: *S. floribunda, S. didymobotrya, S. multiglandulosa, S. sophera, S. obtisfolia, S. occcidentalis, S. obtusfolia, S. siamea, S. alexanderina, S. longiracimosa* and *S. singueana*.

All the reported anthraquinones from *Senna* species (**1-81**) are substituted in both rings A and C. It is well established that anthraquinones (and their derivatives) arising by acetate-malonate pathway are substituted in both rings [16], and it is very likely that all the compounds isolated from *Senna* species arise by acetate-malonate route. Since the compounds isolated from *C. torosa* [16, 17, 33-44] and the ten *Senna* species are structurally similar and share the same pathway, it is possible to catagorize or place *C. torosa* under the genus *Senna*. Recently it has been found that the morphological description of *C. torosa* is identical to that of *S. sophera* [5]. Thus, *C. torosa* is *S. sophera*. This fact indicates the importance of anthraquinones and their derivatives in chemotaxonomic classification in order to support or reject the botanists' classification that is based on morphological variations of plants.

1.5 OBJECTIVE OF THE PROJECT

The objective of this research work is phytochemical investigation of the stem bark of *Senna floribunda* and *Senna didymobotrya* which are two of the *Senna* species that are found in Ethiopia [5].

1.5.1 *Senna floribunda*

Senna floribunda is a woody herb, shrub or small tree 1-5 m high, and distributed in places of 1700-2400 m altitudes. In Ethiopia it is found in Shewa, Arsi, Keffa, Sidamo, Hararghe and Illubabor.

From earlier investigations of the roots, leaves, flowers and pods of this species anthraquinones, flavonoids, bianthraquinones, preanthraquinones, and glycosides of anthraquinones and flavonoids have been isolated [4, 7, 13, 46, 51, 52] (see Tables 1, 2 and 3).

1.5.2 *Senna didymobotrya*

Senna didymobotrya is a bushy shrub 1- 5 m. high distributed in places of 1450-2400 m altitudes. In Ethiopia it grows in Wello, Wellega, Shewa, Arsi and Sidamo.

From earlier investigations of the leaves, pods and fruits of this species anthraquinones, flavonoids, bianthraquinones, preanthraquinones and glycosides of anthraquinones and flavonoids have been isolated [12, 13, 18, 19] (see Tables 1, 2 and 3).

2.0 RESULTS AND DISCUSSION

In this study we have examined the stem bark of *S. floribunda* and *S. didymobotrya*. This resulted in the isolation and characterization of four bianthraquinones and three anthraquinones from the stem bark of *S. floribunda,* and four anthraquinones, two bianthraquinones and one naphthoquinone from the stem bark of *S. didymobotrya.*

2.1 The Stem bark of *Senna floribunda*

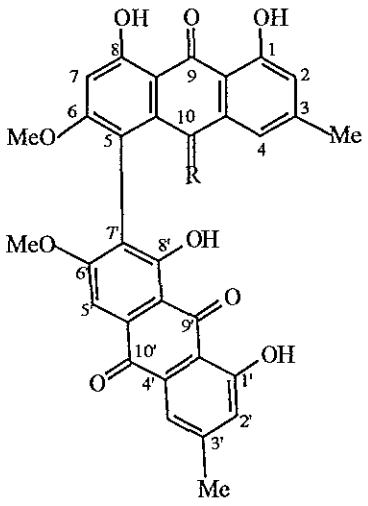
The combined CH_2Cl_2: MeOH. (1:1) and MeOH extracts of the stem bark of *S. floribunda*, after repeated column chromatography on silica gel, Sephadex LH-20, PTLC (see Experimental) resulted in the isolation and characterization of four dimeric anthraquinones: Floribundone-1 (**43**), 5,7'-physcion-fallacinol (**53**), (**83**), (**84**) along with the common anthraquinones: physcion, chrysophanol and emodin.

Chrysophanol, physcion, emodin and floribundone-1 were identified on the basis of their TLC similarity with the authentic samples previously isolated from the leaves and pods of this plant, and by comparing their respective melting points with those reported in the literature [4, 16, 26, 46]. Compounds **83** and **84** were characterized as 5,7'-physcion-physcion anthrone and 5,5'-physcion-physcion anthrone, respectively, and are found to be new bianthraquinones.

2.1.1 5,7'-Physcion-fallacinol (53)

This compound was previously reported as a natural product from the pods of *S. floribunda* [4]. It is a brownish yellow compound that turned red on a TLC plate upon spraying with 5% KOH in methanolic solution. The colour change is characteristic for hydroxyanthraquinones [16]. The UV-Vis spectrum showed bands at 227, 282, and 445 nm which are suggestive of a quinonoid chromophore. The IR spectrum showed bands at 1635 and 1655(sh) cm^{-1} which indicate the presence of chelated and non-chelated carbonyl functional groups. The ^1H NMR spectrum indicated the presence of four chelated hydroxyl groups (δ 13.04, 12.24, 12.16 and 12.06), six aromatic protons (δ 7.78 (*brs*), 7.40 (*brs*), 7.30 (*brs*), 7.04 (*brs*), 7.54 (*s*) and 6.82 (*s*)), two aromatic methoxy groups (δ 3.92 (*s*) and 3.82 (*s*)), one aromatic methyl group (δ 2.36 (*s*)) and a signal at δ 4.82 (*s*) attributable to a $-CH_2OH$ group, suggesting a bianthraquinone based on physcion and fallacinol moieties. This is supported by the MS data. The EIMS showed a peak at *m/z* 582 which accounts for the molecular ion (M^+). Fragment ions that correspond to physcion and fallacinol were observed at *m/z* 284 and 300, respectively. The peaks at *m/z* 284 and 300 arise by cleavage of the internuclear bond of the dimer with the transfer of hydrogen. The two *meta* coupled proton signals at δ 7.78 (*brs*) and 7.30 (*brs*) are assignable to H-4' and H-2', respectively. Other *meta* coupled signals at δ 7.40 (*brs*) and 7.04 (*brs*) are assignable to H-4 and H- 2, respectively. The two singlet signals at δ 7.54 and 6.82 could be assigned to H-5' and H-7, respectively. Thus, absence of signals assignable to H-5 and H-7' led us to conclude that the two monomeric units are linked at C-5 and C-7' giving structure **53**.

The assignment of signals and the proposed structures of this compound are almost identical to 5,7'-physcion-fallacinol previously isolated from pods of *S. floribunda* [4]. The alternative structure 5,7'-fallacinol-physcion which would have the -CH_2OH group at C-3 and the methyl group at C-3' was ruled out by comparing the observed ^1H NMR spectral data with that of floribundone-1(**43**). In the ^1H NMR spectrum of **43** 3-Me and 3'-Me appear at δ 2.35 and 2.45 ppm, respectively. The observed ^1H NMR data showed that the Ar-Me group appears at δ 2.36 ppm indicating that it is attached to C-3 and not to C-3'. If it were at C-3', the corresponding signal would have appeared at about δ 2.45 ppm.



42. R = H_2

43. R = O

Table 4. Comparison of the observed ^{1}H NMR spectral data of **53** with those reported for 5,7'-physcion-fallacinol [4].

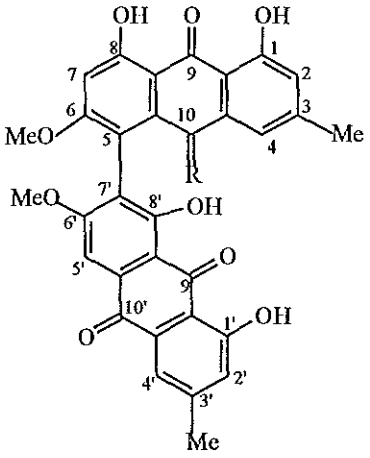
H	Compound **53** (400 MHz, $CDCl_3$) δ (ppm)	5,7'-physcion-fallacinol (300 MHz, $CDCl_3$) δ (ppm)
1-OH	12.06 (*s*)	12.09 (*s*)
H-2	7.04 (*brs*)	7.04 (*brs*)
3-Me	2.36 (*s*)	2.36 (*s*)
H-4	7.40 (*brs*)	7.42 (*brs*)
6-OMe	3.82 (*s*)	3.84 (*s*)
H-7	6.82 (*s*)	6.82 (*s*)
8-OH	12.24 (*s*)	12.25 (*s*)
1'-OH	12.16 (*s*)	12.20 (*s*)
H-2'	7.30 (*brs*)	7.34 (*brs*)
3'-CH_2OH	4.82 (*s*)	4.85 (*s*)
H-4'	7.78 (*brs*)	7.82 (*brs*)
H-5'	7.54 (*s*)	7.57 (*s*)
6'-OMe	3.92 (*s*)	3.89 (*s*)
8'-OH	13.04 (*s*)	13.06 (*s*)

In addition to these ^{1}H NMR spectral data, melting point, IR and UV spectral data are in good agreement with that of 5,7'-physcion-fallacinol reported in literature [4].

2.1.2 5,7'- Physcion-physcion anthrone (83)

This compound is an orange-coloured pigment that turned red on a TLC plate upon spraying with 5% KOH in methanolic solution. This colour change is characteristic for hydroxylanthraquinones [16]. The UV-Vis spectrum showed bands at 232, 365 and 444 nm suggesting a quinonoid chromophore. The IR spectrum showed absorption bands at 3600-3200, 1625 and 1600 cm^{-1} indicating the presence of chelated hydroxyl group, non-chelated and chelated carbonyl functional groups, respectively. The ^1H NMR spectrum revealed the presence of four chelated hydroxyl groups (δ 13.10, 12.37, 12.14 and 12.23 ppm), six aromatic protons (δ 7.38,7.05, 6.87, 6.80, 6.78, and 6.73 ppm), two aromatic methoxy groups (δ 3.83 and 3.86 ppm), two aromatic methyl groups (δ 2.41 and 2.39 ppm) and a peak at δ 3.80 ppm attributable to methylene protons. These observations, together with the molecular ion peak at *m/z* 552 (100%) in the EIMS of the compound led us to the conclusion that compound **83** is a bianthraquinone based on physcion and physcion anthrone moieties. These observations also showed that compound **83** is an isomer of floribundone-2 (**42**) previously isolated from the leaves of this species [46]. This assumption was further supported by the similarity of their molecular ions (M^+) which appeared at *m/z* 552 (100%). But one experimentally observed fact is that **83** is more polar than floribundone-2 (**42**). In addition **83** is more stable towards oxidation whereas floribundone-2 is not stable. It is easily oxidized to floribundone-1 (**43**). So **42** usually exits as a mixture, and it is difficult to get it in a pure form. In the ^1H NMR spectrum of **42** the methyl group attached to the physcion anthrone moiety appears at δ 2.25 ppm. But

in the ^{1}H NMR spectrum of **83** (see Table 4) there is no signal at δ 2.25 ppm. This indicates that the physcion anthrone is not in the upper part of the dimer as that of **42**. So this observation could serve as an additional evidence to show that 83 is different from **42**. There is also a difference in their melting point values.



42. R = H_2

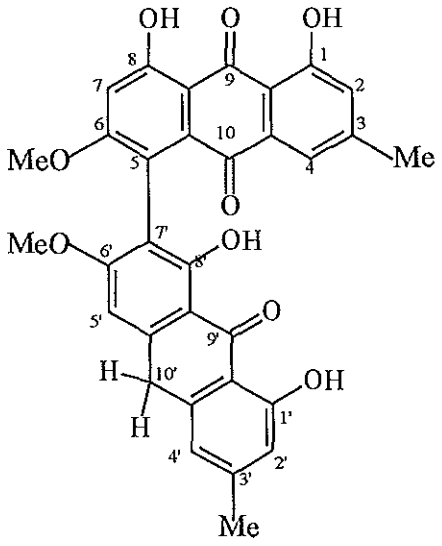
43. R = O

Table 5. ^{1}H NMR data of floribundone-1 (**43**) and floribundone-2 (**42**)

H	**42** (400 MHz, $CDCl_3$)[46] δ (ppm)	**43** (400 MHz, $CDCl_3$)[46] δ (ppm)
1-OH	12.30 (*s*)	12.10 (*s*)
H-2	6.64 (*brs*)	7.04 (*brd*)
3-Me	2.25 (*s*)	2.35 (*s*)
H-4	6.52 (*brs*)	7.42 (*d*)
6-OMe	3.85 (*s*)	3.82 (*s*)
H-7	6.53 (*s*)	6.83 (*s*)
8-OH	12.05 (*s*)	12.05 (*s*)
H-10	3.8-3.9 (*s*)	-------
1'-OH	12.10 (*s*)	12.20 (*s*)
H-2'	7.08 (*brd*)	7.06 (*brs*)
3'-Me	2.45 (*s*)	2.45 (*s*)
H-4'	7.66 (*brd*)	7.65 (*brs*)
H-5'	7.56 (*s*)	7.57 (*s*)
6'-OMe	3.75 (*s*)	3.85 (*s*)
8'-OH	13.10 (*s*)	13.10 (*s*)

From the ^{1}H NMR spectral data of **83** three of the aromatic protons (δ 7.38 (*brs*), 7.05 (*brs*) and 6.80 (*s*)) resonances could be assigned to the physcion moiety of a bianthraquinone system [46].The peaks at δ 7.05 (*brs* or *d-like*), 7.38 (*brs* or *d-like*) and 6.80 (*s*) are assignable to 2-H, 4-H and 7-H, respectively. The observation that the signal

assignable to 7-H of the physcion moiety was a sharp singlet (not broadened), together with the absence of a signal assignable to 5-H, established position C-5 as the point of linkage to the physcion anthrone moiety. The three proton resonances at δ 6.78 (*brs*), 6.73 (*brs*) and 6.78 (*s*) could be assigned to H-2', H-4' and H-5', and absence of the signal assignable to H-7' enabled us to conclude the internuclear bond of the bianthraquinone to be between C-5 and C-7'. Due to the presence of hydrogen bonding caused by carbonyl group at C-10, protons on C-2 and C-4 should have chemical shifts greater than δ 7.0, and those at positions C-2', C-4' and C-5' should resonate below δ 7. So it is impossible to assign δ 7.05 (*brs*) and 7.38 (*brs*) to 2'-H and 4'-H, and 6.87 (*brs*) and 6.73 (*brs*) to 4-H and 2-H, respectively. The proposed structure of **83** is shown below.



83

Table 6. ^{1}H NMR data of **83**

H	**83** (300 MHz, $CDCl_3$) δ (ppm)
1-OH*	12.13 (*s*)
H-2	7.05 (*brs*)
H-4	7.38 (*brs*)
H-7	6.80 (*s*)
8-OH*	12.31 (*s*)
3-Me	2.39 (*s*)
6-OMe	3.83 (*s*)
1'-OH*	12.14 (*s*)
H-2'	6.87 (*brs*)
H-4'	6.73 (*brs*)
H-5'	6.78 (*s*)
3'-Me	2.41 (*s*)
6'-OMe	3.86 (*s*)
10'-H_2	3.80 (*s*)
8'-OH*	13.10 (*s*)

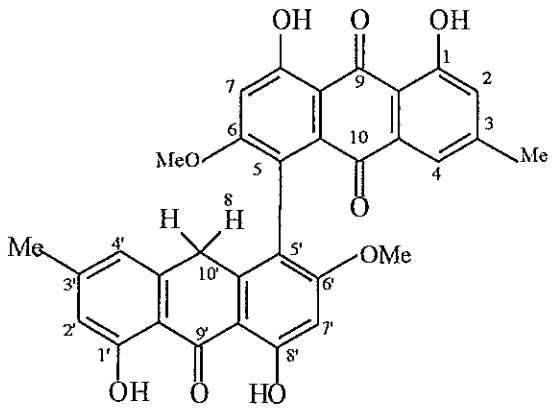
*May be interchanged.

The assignment of δ 2.39 (*s*) to 3-Me was based on comparison with the reported value of 3-Me of floribundone-1 (**43**). If the physcion moiety were in the lower part of the bianthraquinone, the corresponding signal would appear at δ 2.45. There is a small upfield shift for H-4 and 3-Me. Due to the twisted conformation of the dimer, these protons fall in the shielding zone of the lower anthraquinone moiety. So 3-Me has shown a small upfield shift as compared to that of 3'-Me.

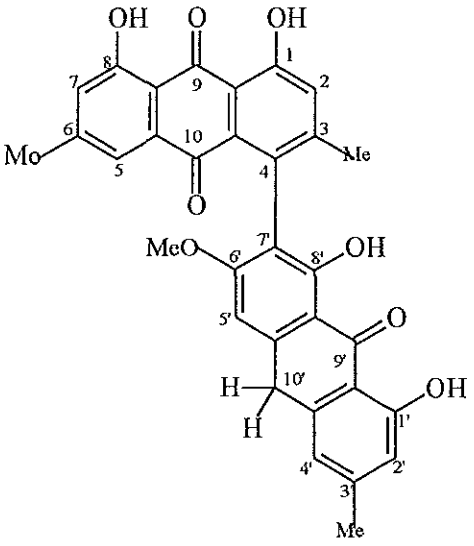
2.1.3 5,5'-Physcion-physcion anthrone (84)

This is an orange coloured compound that turned red on a TLC plate upon spraying with 5% KOH in methanolic solution. This colour change is a positive test for hydroxylanthraquinones [16]. The UV-Vis spectrum showed bands at 234, 360 and 440 nm suggesting a quinonoid chromophore. The IR spectrum showed bands at 3600-3200, 1631 and 1615 cm^{-1} that indicate the presence of chelated hydroxyl group, non-chelated and chelated carbonyl functional groups, respectively. The ^1H NMR spectrum revealed four chelated hydroxyl groups (δ 13.08, 12.33, 12.10, 12.05). The aromatic region displayed six aromatic proton resonances (δ 7.40 (*brs*), 7.04 (*brs*), 6.83 (*s*), 6.81 (*s*), 6.80 (*s*) and 6.73 (*s*)), two methoxy groups (δ 3.84 (*s*) and 3.82 (*s*)) and two aromatic methyl groups (δ 2.40 (*s*) and 2.35 (*s*)). The ^1H NMR data undoubtedly indicate that **84** is a bianthraquinone based on physcion and physcion anthrone moieties. This assumption is supported by the peaks in the EIMS spectrum of **84** at *m/z* 270 and 284 that account to physcion anthrone and physcion moieties, respectively, arising by cleavage of the C-C linkage of the dimer with the transfer of hydrogen. All these observations led us to conclude that compound **84** is most likely an isomer of **83** with two possible alternative structures **84** and **84a**. The H-H COSY spectrum showed that aromatic protons at δ 7.40 and 7.04, and δ 6.80 and 6.73 are coupled with each other. Based on signal assignment of **83**, signals at δ7.40, 7.04, and 6.83 are attributable to H-4, H-2 and H-7 of the physcion moiety, respectively. Due to the effect of 8-OH and 6-OMe which are in *ortho* position to H-7, and exhibit electron donating property, the signal assignable to H-7 should be upfield (<δ 7.0). So it would be impossible to assign the two *meta* coupled protons (δ 7.40 and

7.04) to H-5 and H-7, respectively. They belong to H-4 and H-2, respectively. The proton resonance at δ 6.83 (*s*) is therefore assignable to H-7 and not to H-2. This could indicate that the position of linkage is most likely C-5 and not C-4. Thus, structure **84a** can be ruled out as a possibility. The remaining structure **84** could be the probable structure that could be proposed from the above spectral data.



84



84a

Table 7. ^{1}H NMR spectral data of **84**

H	**84** (300 MHz, $CDCl_3$) δ (ppm)
1-OH*	12.05 (*s*)
H-2	7.04 (*brs*)
H-4	7.40 (*brs*)
H-7	6.83 (*s*)
6-OMe	3.82 (*s*)
3-Me	2.35 (*s*)
8-OH*	12.33 (*s*)
1'-OH*	12.10 (*s*)
H-2'	6.80 (*brs*)
3'-Me	2.40 (*s*)
H-4'	6.73 (*brs*)
6'-OMe	3.84 (*s*)
H-7'	6.81 (*s*)
10'-H_2	3.80 (*s*)
8'-OH	13.08 (*s*)

* May be interchanged.

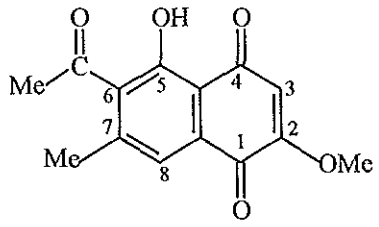
2.2 The Stem Bark of *S. didymobotrya*

Our investigation of the CH_2Cl_2: MeOH (1:1) extract of the stem bark of *S. didymobotrya*, after repeated column chromatography on silica gel, Sephadex LH-20, PTLC (see Experimental) resulted in the isolation of four compounds: **82**, **70**, **6**, **59** along with the common anthraquinones chrysophanol, physcion and emodin. Physcion, chrysophanol and emodin were identified by direct TLC comparison with their respective authentic samples, and by the similarity of their melting points with those reported in literature [16].

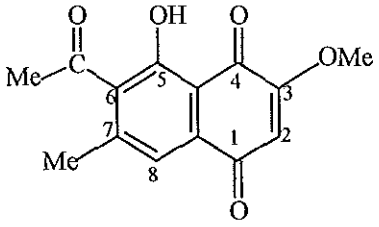
Compound **82** was characterized to be 2-methoxystypandrone, **70** as 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone. Compounds **6** and **59** were characterized as fallacinol and 10,10'-bichrysophanol, respectively.

2.2.1 2-Methoxystypandrone (82)

This is a yellow substance that turned to violet on a TLC plate upon spraying with 5% KOH in methanolic solution. This colour change is suggestive of the presence of chelated hydroxyl group (or *peri*- hydroxyl group). The ^1H NMR spectrum revealed six singlets corresponding to a chelated hydroxyl group (δ 12.50), an aromatic proton (δ 7.52), a benzenoid proton(δ 6.10), a methoxy group (δ 3.94), an acetyl methyl (δ 2.60) and an aromatic methyl (δ 2.35) groups. These spectral data enable us to propose two alternative structures: **82** and **82a**.



82



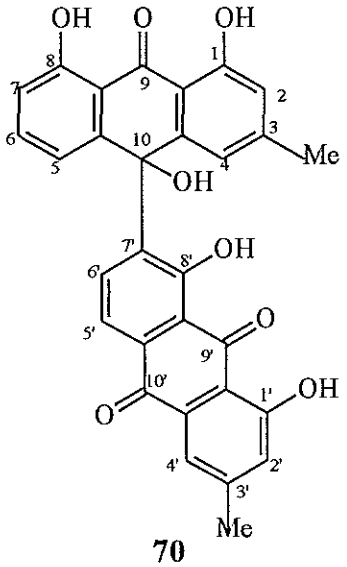
82a

The possibility of structure **82a** is ruled out because the ^{1}H NMR spectrum of orientalone (**82a**) shows six singlets at δ 12.60, 7.65, 6.20, 3.98, 2.65 and 2.39 ppm corresponding to a chelated hydroxyl group, an aromatic proton, a benzenoid proton, a methoxy group, an acetyl methyl and aromatic methyl group, respectively [53]. The reported melting point of **82a** is 195-196°C. The observed ^{1}H NMR spectral data and the measured melting point of **82** are different from those reported for orientalone, and are in good agreement with those reported for 2-methoxystypandrone [17, 30].

2.2.2 10-(Chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (70)

This is an orange-yellow compound that turned red on a TLC plate upon spraying with 5% KOH in methanolic solution, i.e., it shows a charaterstic colour reaction of hydroxy anthraquinones. The ^{1}H NMR spectral data indicated the presence of four chelated hydroxyl groups, nine aromatic protons, two aromatic methyl groups and one non-chelated hydroxyl group (δ 2.80). These observations were helpful to conclude that the compound is a bianthraquinone based on two chrysophanol units.

The four *meta* coupled protons at δ 6.78 (*brs*), 6.60 (*brs*), 7.04 (*brs*), and 7.64 (*brs*) are assignable to H-2, H-4, H-2' and H-4', respectively. The two *ortho* coupled protons at δ 8.64 (*d*) and 8.15 (*d*) are assignable to H-5' and H-6', respectively. The proton signals of an ABC system at δ 6.94 (*d*), 7.41 (*t*) and 6.80 (*d*) could be assigned to H-5, H-6, and H-7, respectively. Since there is no signal at δ 3.8-3.9, the compound has no aromatic methoxy group. The proton signals of H-4 and H-5 are upfield due to the absence of a carbonyl group at C-10. This explains that the monomeric units are linked at C-10 and C-7'. So based on the above information structure **70** was assigned to this compound.



70

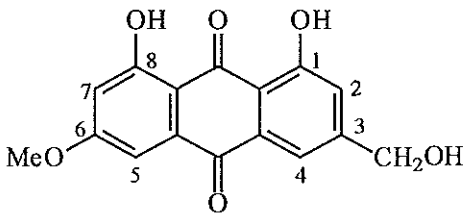
The observed ^{1}H NMR spectral data along with the measured melting point (see Experimental) are in good agreement with those reported in the literature for 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone [17, 30].

Table 8. Comparison of the observed ^{1}H NMR spectral data of **70** with those reported for 10-(Chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone in literature [30].

H	Compound **70** (400 MHz, $CDCl_3$) δ (ppm)	Reported data (400 MHz, $CDCl_3$) δ (ppm)
1-OH	11.75 (*s*)	11.75 (*s*)
H-2	6.78 (*brs*)	6.76 (*d*)
3-Me	2.25 (s)	2.20 (s)
H-4	6.58 (*brs*)	6.58 (*d*)
H-5	6.80 (*d*)	6.78 (*d*)
H-6	7.41 (*t*)	7.38 (*t*)
8-OH	12.15 (*s*)	12.15 (*s*)
1'-OH	12.35 (*s*)	12.35 (*s*)
H-2'	7.04 (*brs*)	7.02 (*d*)
3'-Me	2.45 (*s*)	2.40 (*s*)
H-4'	7.64 (*d*)	7.60 (*d*)
H-5'	8.64 (*d*)	8.64 (*d*)
H-6'	8.15 (*d*)	7.98 (*d*)
8'-OH	12.45 (*s*)	12.45 (*s*)

2.2.3 Fallacinol (6)

This is a yellow pigment that turned red on a TLC plate upon spraying with 5% KOH in methanolic solution. This colour change is indicative of hydroxyanthraquinones. The ^{1}H NMR spectrum showed the presence of two chelated hydroxyl groups (δ 12.30, 12.21), four aromatic protons at δ 7.78 (*brs*), 7.38 (*d*), 7.32 (*brs*) and 6.70 (*d*) that are assignable to H-4, H-5, H-2, and H-7, respectively. The signals at δ 4.82 (*s*) and 3.94 (*s*) correspond to -CH_2OH and -OCH_3, respectively. The observed ^{1}H NMR and UV spectral data along with the measured melting point are in good agreement with those reported for fallacinol in the literature [16,18].

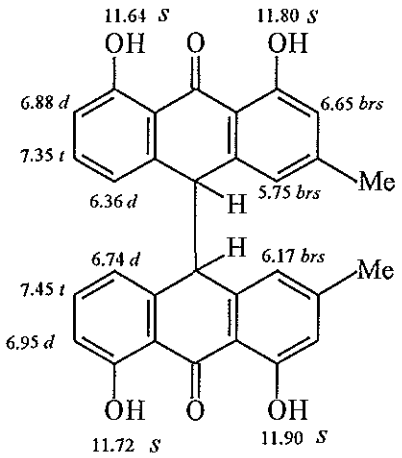


6

2.2.4 10,10'-Bichrysophanol (59)

This is a light yellow compound that turned deep yellow with gradual change to light brown on a TLC plate upon spraying with 5% KOH in methanolic solution. The IR spectrum showed a strong band at 1620 cm^{-1} which indicated the presence of chelated carbonyl functional group. The ^{1}H NMR spectrum showed the presence of four chelated hydroxyl groups (δ 11.90, 11.80, 11.72 and 11.64), ten aromatic protons two aromatic methyl groups and two methine protons at (δ 4.48 (*brs)*). These spectral data

easily led us to conclude that the compound is a symmetrical bianthraquinone made of two chrysophanol anthrone moieties linked at 10,10'-positions.



59

The ^{1}H NMR and IR data along with melting point are in good agreement with those reported for 10,10'-bichrysophanol [30].

3.0 EXPERIMENTAL

3.1 General

Instruments:

^{1}H NMR: 400 MHz spectra were recorded on a Varian VXR-5000 at the Chalmers University of Technology, Gothenburg, Sweden. 300 MHz and 400 MHz spectra were recorded on a Brucker 300 MHz instrument at the University of Botswana, Gaborone, Botswana.

UV: Milton Roy Spectronic 1001plus

IR: Pye Unicam SP2000 IR spectrophotometer

MS: EIMS (low resolution) were recorded at the University of Botswana.

MP: BOCK-MONOSCOP, uncorrected

Chromatography:

Column chromatography: Silica gel 60, particle size 0.063-0.200 mm (70-230 mesh ASTM).

Sephadex LH- 20 ($CHCl_3$:MeOH (2:1))

Analytical TLC: Silica gel 60 PF_{254} (Fluka) coated on aluminium sheets, 0.20 mm thickness.

Preparative TLC: silica gel 60 PF_{254+36}; (Merck) 1 mm, 0.75 mm, 0.50 mm.

Impregnation of silica gel: with 5% oxalic acid.

Spray reagents: 5% KOH in methanolic solution.

Solvent systems:

CH_2Cl_2:MeOH (1:1) (solv. I), CH_3OH (solv. II), $CHCl_3$ (solv. III), $CHCl_3$:MeOH (2:1) (solv. IV), petrol:EtOAc (9:1) (solv. V), $CHCl_3$:EtOAc (4:1) (solv. VI), n-hexane:EtOAc (4:1) (solv. VII), $CHCl_3$:MeOH (19:1) (solv. VIII), $CHCl_3$:MeOH (9:1) (solv. IX), petrol:EtOAc (4:1) (solv. X).

3.1 The Stem Bark of *Senna floribunda*

Plant material: The stem bark of *S. floribunda* was collected from A.A.U., Chemistry Department Garden on August 20, 1998 and was identified by Prof. Sebsebe Demissew, Department of Biology, A.A.U. The specimen is deposited at the National Herbarium with a voucher No W-1.

Extraction and isolation: The dried and ground plant material (500 g) was soaked in 1 L of 5% acetic acid (by volume), and allowed to dry in air. The dried sample was extracted with 3.5 L of solv. I after it has been soaked for 10 hrs, and then with 1 L of solv. II after soaking it for 30 minutes. The combined extract was then concentrated, under reduced pressure, to yield 100 g black gummy residue. A portion of the combined extract (30 g) was subjected to column chromatography that was packed with 340 g silica gel impregnated with 5% oxalic acid, to ease isolation of polar components, and eluted with chloroform to collect 58 fractions: Frs 1-24 100 ml each and frs 25-58 200 ml each. Based on their TLC similarity frs 2-9, frs 10-35, frs 36-58 were combined, and labeled as **B**, **C**, and **D**, respectively. Fr 1 was labeled as **A**.

B was subjected to chromatography over Sephadex LH-20 (solv. IV) to collect six fractions (20 ml each). The first fraction was chlorophyll, and was discarded. The second fraction was applied to PTLC (silica gel, solv. V) to isolate chrysophanol, physcion and floribundone-1 (**43**)

C was chromatographed on Sephadex LH-20 (solv. IV) to collect five fractions (fr 1 10 ml, fr 2 and fr 3 20 ml each, fr 4 20 ml, fr 5 60 ml). The third and fourth fractions were combined and subjected to column chromatography (silica gel, solv. VI) to collect 18 factions (40 ml each). Frs 4 - 18 were combined, and rechromatographed on a column of silica gel (solv. VI) to collect two fractions (fr 1 250 ml and fr 2 150 ml). Then fr 1 was freed of solvent to afford 50 mg of 5,7'-physcion-fallacinol (**53**). The fifth fraction, obtained from Sephadex LH-20 chromatography of **C**, was subjected to a column chromatography (silica gel, solv. VII) to collect five fractions (25 ml each). The second fraction was freed of solvent to yield 34 mg of emodin.

D was subjected to subjected to chromatography over Sephadex LH-20 (solv. IV) to collect three fractions (30 ml each). The second fraction was applied to PTLC (silica gel, solv. VIII, multiple development) to yield 6 mg of **83** and 9 mg of **84**.

3.3 Characterization of compounds isolated from the stem bark of *Senna floribunda*.

5,7'-Physcion-fallacinol (**53**): mp 120-122° C (dec) (lit [4] mp 123-125°C); Uv ($CHCl_3$) λ_{max} : 227, 228, 445 nm; IR ν_{max} (KBr) cm^{-1} :1635 and 1655. EIMS: *m/z* 582 (M^+), 516,

300, 284, 270, 248, 227, 180; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.06 (*s*, 1H, 1-OH), 7.04 (*brs*, 1H, 2-H), 2.36 (*s*, 3H, 3-Me), 7.40 (*brs*, 1H, 4-H), 3.82 (*s*, 3H, 6-OMe), 6.82 (*s*, 1H, 7-H), 12.24 (*s*, 1H, 8-OH), 12.16 (*s*, 1H, 1'-OH), 7.30 (*brs*, 1H, 2'-H), 4.82 (*s*, 2H, 3'-CH_2OH), 7.78 (*brs*, 1H, 4'-H), 7.45 (*s*,1H, 5'-H), 3.92 (*s*, 3H, 6'-OMe), 13.04 (*s*, 1H, 8'-OH).

5,7'-Physcion-physcion anthrone (**83**): mp 184-186°C; UV ($CHCl_3$) λ_{max} : 232, 365, 444 nm; IR ν_{max} (KBr) cm^{-1} : 3600-3200, 1600 and 1625. EIMS: *m/z* 552 (M^+), 534(M^+-H_2O), 521, 503, 297, 270, 227; ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.13 (*s*, 1H, 1-OH), 7.05 (*brs*, 1H, 2-H), 7.38 (*brs*, 1H, 4-H), 6.80 (*s*, 1H, 7-H), 12.31 (*s*, 1H, 8-OH), 12.14 (*s*, 1H, 1'-OH), 6.87 (*brs*, 1H, 2'-H), 6.73 (*brs*, 1H, 4'-H), 6.78 (*s*, 1H, 5'-H), 13.10 (*s*, 1H, 8'-OH), 2.39 (*s*, 3H, 3-Me), 2.41 (*s*, 3H, 3'-Me), 3.83 (*s*, 3H, 6-OMe), 3.86 (s, 3H, 6'-OMe), 3.80 (*s*, 2H, 10'-H_2).

5,5'-Physcion-physcion anthrone (**84**): mp 181-182°C; UV ($CHCl_3$) λ_{max} : 234, 360, 440 nm: IRν_{max} (KBr) cm^{-1}: 3600-3200, 1631 and 1615: EIMS: *m/z* 284 (100%), 270, 255, 227; ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.05 *(s,* 1H, 1'-OH), 7.04 (*brs*, 1 H, 2-H), 7.40 (*brs*, 1H, 4-H), 6.83 (*s*, 1H, 7-H), 3.82 (*s*, 3H, 6-OMe), 2.35 (s, 3H, 3-Me), 12.33 (*s*, 1H, 8'-OH), 12.10 (*s*, 1H, 1'-OH), 6.80 (*brs*, 1H, 2'-H), 2.40 (*s*, 3H, 3'-Me), 6.73 (*brs*, 1H, 4'-H), 3.84 (*s*, 3H, 6'-OMe), 6.81 (*s*, 1H, 7'-H), 13.08 (*s*, 1H, 8'-OH), 3.80 (*s*, 2H, 10'-H_2).

3.4 The Stem Bark of *Senna didymobotrya.*

Plant material: The stem bark of *S. didymobotrya* was collected from A.A.U., Chemistry Department Garden on August 20, 1998 and was identified by prof. Sebsebe Demissew, Department of Biology. The specimen is deposited at National Herbarium with a voucher No AH-2 &3.

Extraction and isolation: The dried and ground plant material (520 g) was soaked in 1.2 L of 5% acetic acid, and allowed to dry. The dried material was soaked in petrol 2 L overnight, and washed with 3 L petrol. The marc was then soaked in 2 L of solv. I overnight, and washed again with 1.5 L of solv. I. The extract was concentrated to yield 96 g brownish gummy residue.

A portion of the extract (30 g) was adsorbed on 30 g silica gel and was applied to a column packed with 300 g silica gel (impregnated with 5% oxalic acid). The column was then eluted with only solv. III to collect 18 fractions (each 150 ml). The first fraction was oily and discarded. The second fraction was labeled as **E**, frs 3-11 were combined based on their TLC similarity, and were labeled as **F.** Frs 12-14 and frs 15-18 were also combined based on their TLC similarity, and labeled as **G** and **H**, respectively.

E was subjected to chromatography over Sephadex LH-20 (solv. IV) to collect three fractions (50 ml each). Frs 2-3 were combined and applied to PTLC (silica gel, solv. V) to yield chrysophanol and physcion.

F was chromatographed over Sephadex LH-20 (solv. IV) to collect five fractions (50 ml each). Frs. 3-4 (**I**), combined based on their TLC similarity, showed the presence of three compounds. Then **I** was applied to small column of silica gel (solv. X) to collect fourteen fractions (50 ml each). Frs 5-14 were combined and subjected to repeated PTLC (silica gel, solv. VI, solv. IX, solv. X) to yield 2-methoxystypandrone (**82**), 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (**70**), 10,10'-bichrysophanol anthrone(**59**).

G was subjected to Sephadex LH-20 (solv. IV) to collect six fractions (35 ml each). Frs 4-5 (**J**) were combined on the basis of their TLC similarity. The sixth fraction (**K**) showed one major spot of anthraquinone. Then **K** was rechromatographed over Sephadex LH-20 (solv. IV) to collect two fractions (50 ml each). The second fraction (**L**) was then subjected to a small column of silica gel (solv. IX) to collect six fractions (50 ml each). Frs 2-6 were combined and concentrated to yield 20 mg of pure compound which was identified to be emodin by TLC comparison with authentic sample. Fr **J**, after repeated chromatography over Sephadex LH-20 (solv. IV), yield fallacinol (**6**).

3.5 Characterization of compounds from the stem bark of *Senna didymobotrya.*

2-Methoxystypandrone (**82**): R_f 0.32 (solv. III), 0.35 (solv. X); mp 183-185°C, (lit [30] mp 187-188°C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.50 (*s*, 1H, 5-OH), 6.10 (*s*, 1H, 3-H), 3.94 (*s*, 3H, 2-OMe), 2.35 (s, 3H, 7-Me), 7.52 (s, 1H, 8-H), 2.60 (*s*, 3H, -COMe).

10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (**70**): R_f 0.26 (solv. III), 0.54 (solv. X); mp 227-229.5° C (lit [30] mp 224-226°C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.75 (*s*, 1H, 1-OH), 6.78 (*brs*, 1H, 2-H), 2.25 (*s*, 3H, 3-Me), 6.60 (*brs*, 1H, 4-H), 6.80 (*d*, 1H, 5-H), 7.41 (*t*, 1H, 6-H), 6.94 (*d*, 1H, 7-H), 12.15 (*s*, 1H, 8-OH), 12.35(*s*, 1H, 1'-OH), 7.04 (*brs*, 1H, 2-H), 2.45 (*s*, 3H, 3'-Me), 7.64 (*brs*, 1H, 4'-H), 8.64(*d* 1H, 5'-H), 8.15 *d* (1H, 6'-H), 12.45 (*s*, 1H, 8'-OH).

Fallacinol (**6**): R_f 0.17 (solv. III), 0.15 (solv. X); mp 242-243°C, (lit [16] mp 245-247°C); UV ($CHCl_3$) λ_{max} : 234, 280, 440 nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (*brs*, 1H, 4-H), 7.38 (*d*, 1H, 5-H), 7.32 (*brs*, 1H, 2-H), 6.70 (*d*, 1H, 7-H), 4.82 (*s*, 2H, 3-CH_2OH), 3.94 (*s*, 3H, 6-OMe), 12.30 (*s*, 1H, 8-OH), 12.21 (*s*, 1H, 1-OH).

10,10'-bichrysophanol (**59**): R_f 0.68 (solv. III), 0.72 (solv. X); mp 202-205°C (lit [52] mp 208-210°C); IR ν_{max} (KBr) cm^{-1}: 1620; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.64 (*s*, 1H, 8-OH), 6.88 (*d*, 1H, 7-H), 7.35 (*t*, 1H, 6-H), 6.36 (*d*, 1 H, 5-H), 4.48 (*d-like*, 2H, 10-H and 10'-H), 5.75 (*brs*, 1H, 4-H), 2.20 (*s*, 3H, 3-Me), 6.65 (*brs*, 1H, 2-H), 11.90 (*s*, 1H, 1'-OH), 6.72 (*brs*, 1H, 2'-H), 2.32 (*s*, 3H, 3'-Me), 6.17 (*brs*, 1H, 4'-OH), 6.74 (*d*, 1H, 5'-OH), 7.45 (*t*, 1H, 6'-OH), 6.95 (*d*, 1H, 7'-H), 11.72 (*s*, 1H, 8'-OH).

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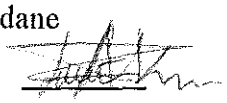
DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Legesse Adane

Signature: 

August, 1999