

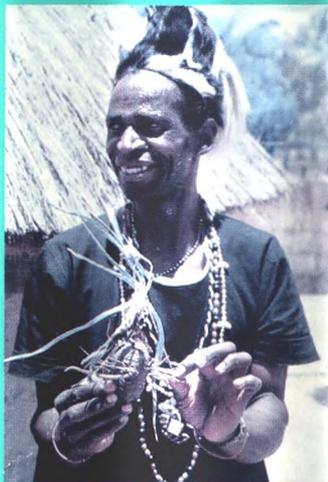


International Organization for Chemical  
Sciences in Development

**Working Group on Plant Chemistry**

**CHEMISTRY, BIOLOGICAL AND  
PHARMACOLOGICAL PROPERTIES OF  
AFRICAN MEDICINAL PLANTS**

Proceedings of the first International IOCD-Symposium  
Victoria Falls, Zimbabwe, February 25-28, 1996



Edited by

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**UNIVERSITY OF ZIMBABWE PUBLICATIONS**

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*Institut de Pharmacognosie et Phytochimie, Université de Lausanne, BEP, CH-1015  
Lausanne, Switzerland and Department of Pharmacy, University of Zimbabwe,  
P.O. Box M.P. 167, Harare, Zimbabwe*

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African traditional healer and *Harpagophytum procumbens* (Pedaliaceae)  
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## 8. Quinones and other phenolic compounds from marketed African plants

B.M. ABEGAZ<sup>1</sup>, G. ALEMAYEHU<sup>2</sup>, T. KEBEDE<sup>2</sup>, D. MAHAJAN<sup>1</sup>  
AND M. M. NINDI<sup>1</sup>

<sup>1</sup>Department of Chemistry, University of Botswana, Private Bag 0022, Gaborone, Botswana and <sup>2</sup>Department of Chemistry, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia

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### Introduction

Almost all traditional markets in Africa have sections where plants are sold for a variety of uses. A closer look at even modern shopping centers will reveal that there are thriving businesses of native plants. These uses include medicinal, culinary, fragrance, majico-medical, etc. In each region one finds indigenous plants that have emerged from the local culture and tradition as established items of commerce for that particular community. It is worth noting that many clients have established the utility and efficacy of these plants out of personal previous experiences and so simply proceed to buy them in very much the same way as one would buy common over-the-counter drugs and other personal hygiene aids.

We have been studying plants that are sold in African markets. We have conducted surveys in such markets in several countries in Africa, especially in Ethiopia, Kenya, Uganda, Tanzania (Abegaz and Demissew 1992) and Botswana. This report will deal with our recent findings in which we have identified novel anthraquinone and naphthalene glucosides from *Rhamnus prinoides* (Rhamnaceae) and bianthraquinone pigments from *Senna* (Fabaceae) species.

### *The genus Rhamnus*

The genus *Rhamnus* has been widely investigated. The most comprehensive review of the family being that of Hegnauer (1973). As many as 24 species have been described in the chemical literature. The genus has been a source of a variety of flavonoids which are based on quercetin and kaempferol and their glycosides. Several anthrones, anthraquinones and their glycosides have also been reported. The well known cathartic drug, Cascara bark, is derived from the Asian *R. purshiana* DC Bark (Tyler *et al.* 1988). This plant has been investigated extensively and several C-10 glycosides of emodin (cascarosides A-D), O,C-

diglucosylanthrones (cascarosides E and F) (Mannito *et al.* 1993, 1995) as well as the diastereomeric 10-hydroxyaloins A and B (Rauwald *et al.* 1991) have been reported. There are also several phytochemical reports on Asian *R. formosana* (Kalidhar 1992; Lin and Wei 1993), *R. wightii* (Peppalla *et al.* 1991) and *R. nakahari* (Lin and Wei 1994) revealing the presence of many anthraquinones and naphthalenic derivatives. 6-O-Rhamnosides of emodin (known as frangulin A, B, and glucofrangulin) have also been found in *R. fallax* (Kinet 1967).

### *Rhammus prinoides*

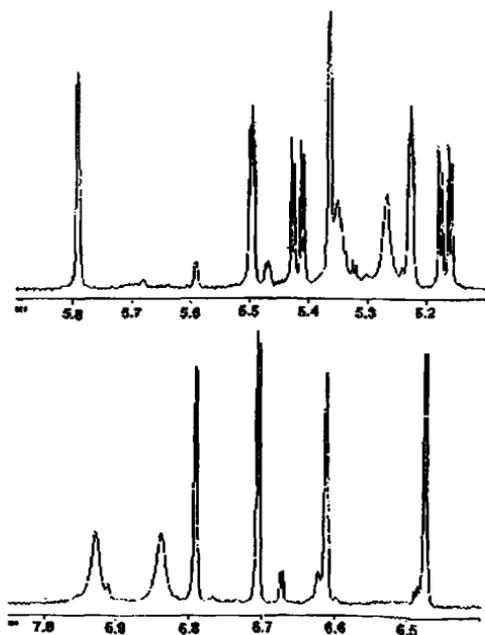
In Africa, the genus *Rhammus* is represented by only two taxa, namely - *R. prinoides* and *R. staddo*. *R. prinoides*, known in Ethiopia by the Amharic name: *Gesho*, is a plant which grows up to 6 meters. It is also known to occur in Cameroon, Sudan, throughout East Africa to South Africa, Angola and in Arabia (Thulin 1989). It is cultivated in Ethiopia, specially in Tigrai, in North Shoa around Kara Kori and Sebeta, just west of Addis Ababa. *Gesho* is an important commodity and is sold in almost every traditional market in Ethiopia.

The leaves and stems of *Gesho* are indispensable ingredients in the making of the traditional fermented beverages *Tella* and *Tej*. In doing so, care is always taken to remove the fruits of the plant from the leaves and stems. The fruits are, however, used for the treatment of ring worm infections. *Tella* is a malt beverage, like beer. *Tej* is also a fermented beverage based on honey. Over 5 million people consume these beverages everyday in Ethiopia. Although it is generally known that *Gesho* imparts the characteristic bitterness of these beverages, more precise understanding of the scientific role of this plant in this traditional brewing process is emerging only very slowly. The first scientific report on *R. prinoides* is that of Salgues (1962) who described the presence of inorganic cations, organic acids and the flavonoid derivative rhamnctin. He also claimed that the leaf extract was toxic to rabbits. The role of *Gesho* in the fermentation process has been investigated (Kleyn and Hough 1971; Sahle and Gashe 1991) and it is claimed that the plant regulates the microflora responsible for the fermentation process. These reports indicate further that the bitterness of the brew is directly related to the amount of *Gesho* added. It has also been reported that extracts from *Gesho* can be used as a commercial hopping agent for beer (Tessema 1994).

### *Secondary metabolites of the fruits of R. prinoides*

Emodin, physcion, emodinanthrone, emodinbianthrone, rhamnazin and prinoidin (1), a novel anthrone rhamnoside diacetate were reported by Abegaz and Dagne (1988). Subsequently the isolation of minor pigments of the fruits, other mono- (6), di- (3, 4, 5), and triacetates (2) of emodin were reported (Abegaz and Peter 1995). It is not clear if these isomeric acetates are true natural products or artefacts formed during preparative thin-layer chromatography on silica gel. However,

solutions of these compounds in chloroform are indefinitely stable in the NMR tube. In earlier work we had isolated a dimer of prinoidin which we initially thought was a natural product. We have now made the observation that the amount of this dimer increases with prolonged contact of the natural products to silica gel during flash chromatography. We are, therefore, inclined to conclude that this dimer is an artefact. The structure of the dimer (7), was nevertheless characterized. FABMS clearly indicated the molecular ion at 970, which is consistent with a dimer of prinoidin. The 600 MHz  $^1\text{H-NMR}$  showed the presence of four chelated hydroxyl signals and eight aromatic proton resonances at  $\delta$ 6.93, 6.84, 6.79, 6.71, 6.61, 5.48, 5.35 and 5.27 (See Table 8.1). The striking feature of the spectrum is the highly shielded aromatic protons at 5.35 and 5.27 ppm. Further more, these two signals and two others at 6.93, 6.84 are significantly broadened as shown in Fig. 8.1. The two shielded signals are assigned to the 5 and 5' protons which are most probably forced out of the plane of the ring by the adjacent sugar substituent on each of the anthraquinone moiety. The reason for the broadening of the four signals is not entirely clear and we are not sure if they are caused by a scissoring type dynamic behaviour. The proton signals of one of the rhamnose moiety is also more shielded. The type of conformation which would be consistent with the observed spectrum is at the present time unclear.



**Fig. 8.1.** Partial  $^1\text{H-NMR}$  Spectrum of dimer 7 (600 MHz) showing aromatic proton signals.

Table 8.1. <sup>1</sup>H-NMR spectral data for dimer 7 (600 MHz, CDCl<sub>3</sub>, δ ppm)

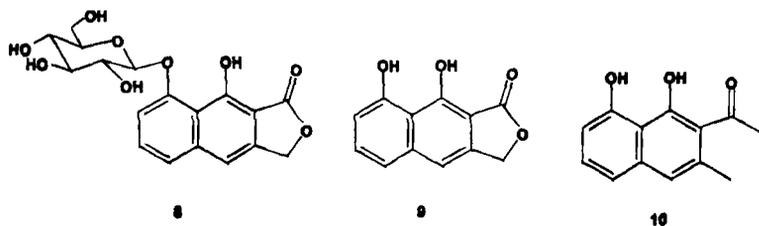
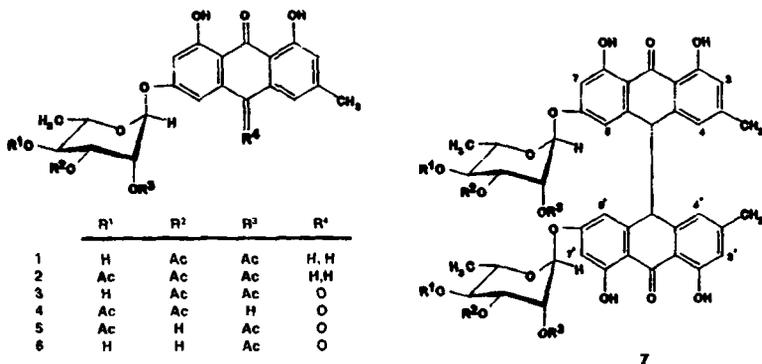
Protons	δ(ppm); J (Hz)	Sugar protons	δ(ppm); J (Hz)
H-2	6.79 <i>s</i>	H-1''	5.79 <i>d</i> (1.40)
H-4	6.93 <i>brs</i>	H-2''	5.50 <i>dd</i> (1.79, 3.49)
H-5	5.35 <i>brs</i>	H-3''	5.42 <i>dd</i> (3.51, 10.04)
H-7	6.71 <i>d</i> (2.30)	H-4''	3.65 <i>m</i>
H-10	4.48 <i>d</i> (3.89)	H-5''	3.95 <i>m</i>
C3-Me	2.48 <i>s</i>	C5''-Me	1.43 <i>d</i> (6.06)
H-2'	6.61 <i>s</i>	H-1'''	5.36 <i>d</i> (1.78)
H-4'	6.84 <i>brs</i>	H-2'''	5.23 <i>dd</i> (1.96, 3.41)
H-5'	5.27 <i>brs</i>	H-3'''	5.17 <i>dd</i> (3.46, 9.87)
H-7'	6.45 <i>d</i> (2.30)	H-4'''	3.65 <i>m</i>
H-10'	4.45 <i>d</i> (3.88)	H-5'''	3.75 <i>m</i>
C3'-Me	2.12 <i>s</i>	C5'''-Me	1.33 <i>d</i> (6.20)
-OH	12.43 <i>s</i>	CO-Me	2.22 <i>s</i>
-OH	11.90 <i>s</i>	CO-Me	2.15 <i>s</i>
-OH	11.81 <i>s</i>	-COMe	2.14 <i>s</i>
-OH	11.64 <i>s</i>	-CO-Me	2.13 <i>s</i>

### Secondary metabolites of the leaves of *R. prinoides*

We have identified 11 secondary metabolites from the leaves. These include the known anthracene derivatives: chrysophanol, physcion and emodin; the flavonoids rhamnocitrin, rhamnezin, quercetin and 3-O-methylquercetin; and the naphthalenic derivatives sorigenin (9), musizin (10) and the previously unknown β-sorigenin-8-O-β-D-glucoside, (geshoidin, 8). An organoleptic evaluation on the above compounds revealed that geshoidin is the most significant bitter substance of the leaves. 3-O-Methylquercetin also displayed bitterness. Further organoleptic evaluation was made by five volunteers who independently confirmed that geshoidin possesses bitter properties. It is interesting to note that geshoidin is bitter despite the presence of a glucose moiety in the structure. The aglycone, sorigenin (9) is in fact not bitter at all. The toxicity of geshoidin to brine shrimp (*Artemia salina*) was evaluated at seven different concentrations over a range of 64 folds (15 to 1000 µg/ml)\*. No lethality was observed. Preliminary assay for possible cytotoxicity of geshoidin has also been negative\*. Although rigorous toxicity tests should be conducted on geshoidin, the results obtained so far suggest that this compound may have commercial potential. The critical question in determining the structure of geshoidin was to provide evidence to show that the

\* We are grateful to Ato Mesfin Bogale of the Faculty of Science, Addis Ababa University for the brine shrimp assay and to Dr. R. Becker of the University of the North for the cytotoxicity assays.

sugar moiety was attached to the 8-position and not at the alternative C-9 position. In fact the alternative structure is known and had been reported from *R. wightii* (Peppalla 1991). The identification of geshoidin was based on spectroscopic and chemical evidence (Abegaz and Kebede 1995). The EI as well as CIMS of geshoidin failed to show a molecular ion. But ESIMS yielded  $[M+23]^+$  ion at 401.



Collision induced dissociation of this parent ion by application of an offset voltage of 35V resulted in the appearance of an ion at  $m/z$  of 239 which is believed to arise by loss of the glucose unit from the  $[M+23]^+$  ion. The co-occurrence of the two naphthalenic compounds **9** and **10** in the leaves suggests a biogenetic relationship of these two compounds and geshoidin (**8**). It seems very probable that musizin (**10**) undergoes oxidative cyclization to sorigenin (**9**) and glucosylation at position 9 to yield geshoidin (**8**).

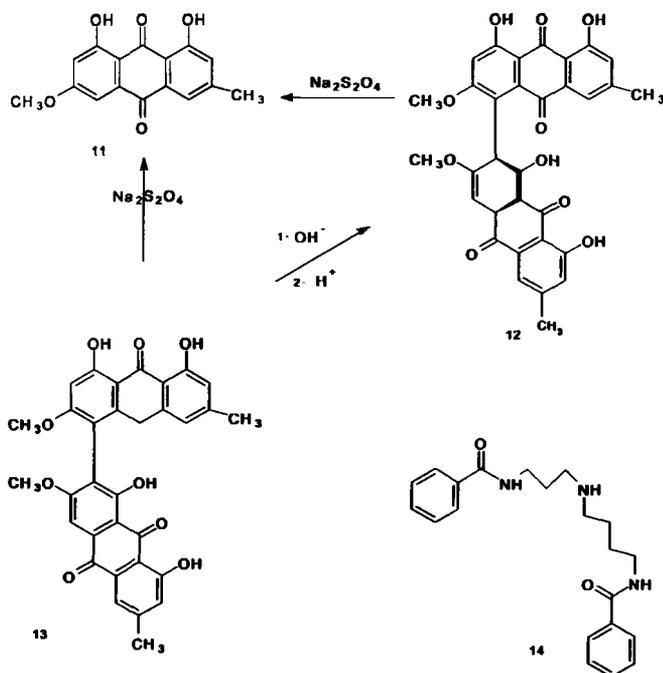
### The genus *Senna*

*Senna* is an important genus which has yielded important purgative drugs. The most famous is *Cassia senna* (*C. acutifolia*), known as Alexandrian senna or *Cassia angustifolia*, also known as Tinnevely senna, or a mixture of the two species. The biologically active constituents of *Senna* are the hydroxyanthracene glycosides known as sennosides. Many members of *Senna* have for a long time

been considered in a broader classification together with the now separate genera of *Cassia* and *Camechrista* (Thulin 1989). We have studied four taxa, namely: *S. didymobotrya*, *S. septemtrionalis*, *S. longiracemosa* and *S. multiglandulosa* and have identified several anthraquinones, anthrones, preanthraquinones, and novel bianthraquinones.

*Senna septemtrionalis* (synonyms: *Cassia laevigata*, *C. floribunda*, *S. floribunda*)

This legume has been shown to contain 8-mono- and digalactosides of physcion as well as chrysophanol and emodin (Singh *et al.* 1980). Our studies on the leaves yielded the common anthraquinones, emodin and physcion, in addition to two novel bianthraquinone pigments for which the names floribundone-1 (**12**) and floribundone-2 (**13**) were given (Alemayehu *et al.* 1988). These compounds represented the second set of examples of an anthraquinone dimer with a 5-7'-bianthracene linkage. The mixture of floribundone-1 and 2 was difficult to separate and it was observed that a solution of floribundone-2 (**13**) was easily oxidized to floribundone-1 (**12**).

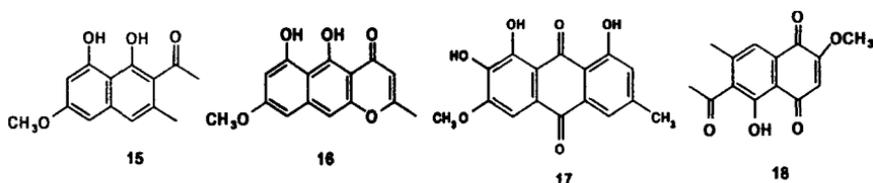


The mixture of the two compounds was also cleaved to physcion by reaction with sodium dithionite. The reductive cleavage of floribundone-2 (**13**) presumably

yields physcion anthrone which would be oxidized to physcion (11) during work up. Alternatively and most probably, the oxidation of floribundone-2 (13) to floribundone-1 (12) may take place faster than the cleavage to yield physcion anthrone directly. Also isolated from the leaves was a  $N^1, N^8$ -dibenzoyl-spermidine (14) and other traces of pigments which at present are unidentified. Floribundone-1 (12) has since then been reported from Mexican *Senna* species (Barba *et al.* 1993) and its atropi-isomer from *Cassia torosa* of Japanese origin (Kitanaka and Takido 1995).

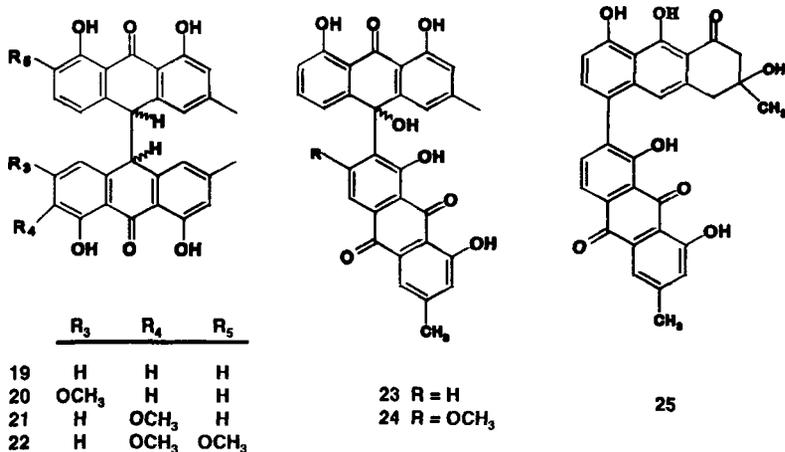
*Senna longiracemosa* (synonym: *C. longiracemosa*)

The leaves yielded chrysophanol, physcion, torachryson (15), rubrofusarin (16), nataloe-emodin (17), 10,10'-bichrysophanol (19), 10,10'-chrysophanol-physcion (20), 10,10'-chrysophanol-isophyscion (21), 10,10'-biisophyscion (22) and 10-hydroxy-10,7'-(chrysophanol-anthrone)-chrysophanol (23) (Alemayehu *et al.* 1993). Compounds 17, 20, 21, and 22 were reported for the first time and 23 had not been reported from the genus *Senna* previously. The root bark also yielded 2-methoxy-stypandrone (18) in addition to chrysophanol, physcion, emodin, 19, 20, 21, and 22 (Alemayehu 1989).

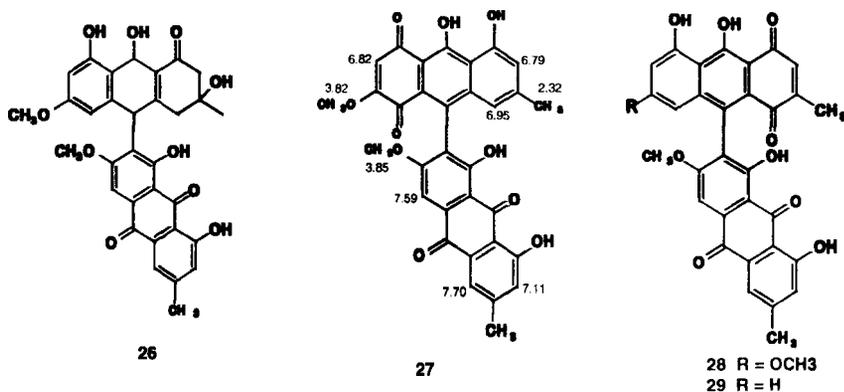


*Senna multiglandulosa* (synonyms: *Cassia multiglandulosa*, *S. tomentosa*, *C. tomentosa*)

Our earlier investigation of the leaves and stems had yielded chrysophanol, emodin, physcion and four bianthraquinones: floribundone-1 (12), torosanin-9,10-quinone (25), anhydrophlegmacin-9,10-quinone (26) and the novel 1,4-quinone: 9-(physcion-7-yl)-5,10-dihydroxy-2-methoxy-7-methyl-1,4-anthraquinone (27) (Abegaz *et al.* 1994). Torosanin-9,10-quinone had been reported as an oxidation product of torosanin obtained from the unripe seeds of *Cassia torosa* (Kitanaka and Takido 1982). The most interesting compound obtained from this plant is the 1,4-quinone 27 for which the name sengulone is proposed. This is the first example of an anthraquinone dimer containing a 1,4-quinone moiety. We were initially intrigued by the possibility of another structure (28) which closely fitted the spectroscopic data for compound 27. Difnoe experiments unequivocally established that the compound isolated from the leaves was 27 and not 28. Thus,



irradiation of the Me signal at  $\delta$  2.49 led to enhancement of the Ar-H signals at 7.70 and 7.11, and irradiating the other Me signal at  $\delta$  2.32 led to the enhancement of two Ar-H signals at 6.95 and 6.79. On the other hand, irradiation of the methoxy signals at 3.82 and 3.88 each led to a corresponding increase in the signals of only one quinonoid proton at 6.20 and 7.59, respectively. These noe data enabled us to reject structure **28** in favour of **27** for the structure of sengulone. Survey of the literature indicates that 1,4-anthraquinones have not been reported from higher plants. There are, however, a few 1,4-anthraquinones isolated from *Aspergillus cristatus* (Laatsch and Anke 1982).

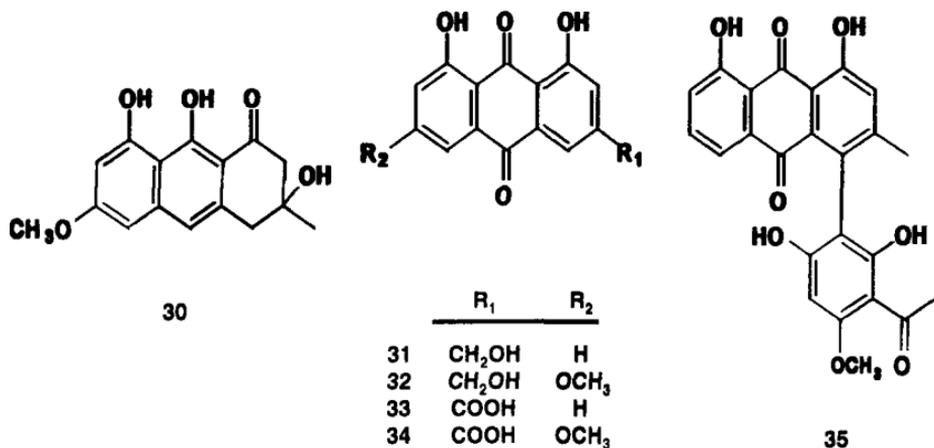


In a subsequent study we undertook to examine the chemical constituents of the seeds of *S. multiglandulosa* and we were able to identify an isomer of sengulone (named iso-sengulone) which fits the rejected alternative structure described above (**28**) in addition to other pigments, namely physcion, torosachryson (**30**),

the bianthraquinones floribundone-1 (12) and anhydrophlegmacin-9,10-quinone (26). Difnoe data were consistent with the structure assigned for 28 as were homonuclear 2D-NMR measurements (COSY45) (Alemayehu and Abegaz 1996).

*Senna didymobotrya* (synonym: *Cassia didymobotrya*)

The leaves yielded the monoanthracene derivatives, chrysophanol, physcion, aloe-emodin (31), fallacinol (32), rhein (33), parietinic acid (34) and the preanthraquinone, torosachryson (30). The isolation of fallacinol (32) and parietinic acid (34) constituted the first report on the occurrence of these two substances from higher plants (Alemayehu *et al.* 1989). Previously they had been reported from lichens and cultures of *Eurotium echinolatum* (Thomson, 1971).



Chrysophanol, aloe-emodin and rhein had been reported earlier from this plant by Egyptian workers (El-Sayyad and Ross 1983). Further examination of the pods of this plant has resulted in the isolation of the common anthraquinones, chrysophanol, emodin and physcion and the known but novel compound knipholone (35). Knipholone which contains an acetylphloroglucinol methyl ether moiety attached to C-4 of chrysophanol was first isolated by Dagne and Steglich (1984) from *Kniphofia foliosa*. Although it has been reported in a number of *Kniphofia* and *Bulbine* species since then (Van Staden and Drewes 1994), this is the first report of this unique anthraquinone in the family Fabaceae. In addition the pods also yielded two new bianthraquinones (24 and 29). Compound 29 constitutes the third example of 1,4-quinone in the genus *Senna* (Alemayehu *et al.* 1996).

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Under the auspices of **The International Organization for Chemical Sciences in Development (IOCD)**, the Working Group on Plant Chemistry chose Victoria Falls in Zimbabwe, to be the site of the first international scientific meeting organized by IOCD. The aim was to bring together scientists from Africa and the international community who are involved in the phytochemical and pharmacological investigation of African medicinal plants.

This volume is a compilation of contributions from both Africa and the rest of the world, and represents an update of different aspects and results of research on the phytochemistry and pharmacology of African medicinal plants.

There is great interest in this field, especially in order to give a scientific basis to the information obtained from traditional healers and ethnomedicine. In some cases, the investigation of the pharmacological and phytochemical aspects of plant preparations can lead to the discovery of natural compounds of high interest, for example those exhibiting anti-cancer and anti-HIV activity which may be of potential as the source for new drugs. Furthermore, these investigations could lead to the development of commercially available traditional medicines in Africa. Thus, the contents of this book should be of use in developing countries where the practice of orthodox medicine is available but beyond the reach of many.

New techniques involved in the procedure of isolation and structure elucidation or pharmacological evaluation of biologically active natural products are also highlighted in this book. Thus this interdisciplinary overview of the latest advances and trends in the field of active compounds of African plant origin should be of general interest to all botanists, chemists, phytochemists and pharmacologists from Africa and elsewhere.





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