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Novel xanthones from *Securidaca longepedunculata* with activity against erectile dysfunction

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ABSTRACT

Aim of study: Securidaca longepedunculata is used in the treatment of erectile dysfunction in South Africa. The aim of the study was to isolate and identify the active constituents and to determine their activity in the relaxation of corpus cavernosal smooth muscle.
Materials and methods: Bioassay guided isolation of the bioactive compounds using a smooth muscle relaxation bioassay and structural elucidation was carried out using different spectroscopic techniques including 2D NMR.
Results: Two new xanthones were isolated; one of them showed potent activity to relax the corpus cavernosal smooth muscle by 97 % in comparison to sildenafil (Viagra) at 1.8×10^{-5} mg/ml.
Conclusions: S. longepedunculata's xanthones stimulate the relaxation of corpus cavenosum smooth muscle, which supports the traditional use of its root bark.

1. Introduction

Traditional medicine still plays a significant role in the lives of many people, and particularly so in the rural Venda area (Limpopo Province, South Africa) (Mabogo, 1990), despite all the new advances in western medicine. In this region several plants are used in the treatment of erectile dysfunction (Rakuambo et al., 2006). Erectile dysfunction was defined as the inability to achieve and maintain an erection sufficient for satisfactory sexual performance during a NIH Consensus Conference (NIH, 1993). Sexual dysfunction in males has found increasing attention partly because of the rapidly ageing populations of the developed countries. The proliferation of medical treatment approaches (particularly PDE-5 inhibitors) for erectile dysfunction was another stimulus for conducting numerous recent epidemiological studies (Beutel et al., 2006). Sexual dysfunction is a multi-disciplinary subject between the specialities of andrology, urology, psychosomatic medicine and psychiatry often requiring an interdisciplinary treatment approach. Based on the sexual response cycle, sexual dysfunction is differentiated according to the phases of desire (hypoactive sexual desire disorder/sexual aversion), arousal (male erectile disorder), ejaculation/orgasm (male orgasmic disorder, premature ejaculation) and resolution (Beutel et al., 2006). In an epidemiological study by Rosen et al. (2003) on the distress caused by erectile dysfunction, erection problems were rated as bothersome by 77.6% of respondents, the degree of bother increasing with the presence of lower urinary tract symptoms, but decreasing with age. Concerns about losing erectile function were reported by 80.0% of the men with no or mild erectile problems. Concerns about erectile function strongly declined with age; the concerns were least above 70% (Rosen et al., 2003; Beutel et al., 2006).

Securidaca longepedunculata Fresen. (Polygalaceae), *Wrightia natalensis* Stapf (Apocynaceae) and *Rhoicissus tridentata* (L.f.) Wild and R.B. Drumm. (Vitaceae) are African medicinal plants (from the Venda region) which are used to treat erectile dysfunction. All three species were previously tested for smooth muscle relaxation properties and *Securidaca longepedunculata* was found to be the most active (Rakuambo et al., 2006). Bioassay guided fractionation led to the isolation of 2-hydroxy-1,7-dimethoxy-xanthone (previously isolated from this species) and 1,4-dihydroxy-7-methoxyxanthone (previously isolated from *Vismia guaramirangae* Huber) (Rakuambo et al., 2004). The former showed good activity on the relaxation of rabbit corpus cavernosum smooth muscle. This was the first report of a xanthone with smooth muscle relaxation properties (Rakuambo et al., 2004). The only other report of experimental work (which we are aware of) on activity against erectile dysfunction from a South African plant species was by Drewes et al. (2002) who isolated active pyrano-isoflavonoids from

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Eriosema kraussianum N.E. Br (Papilionaceae). In a short review by Drewes et al. (2003) they describe the use of several natural products and extracts (not from South Africa) which are currently used to treat erectile dysfunction.

Securidaca longepedunculata is widely used in African traditional medicine as a general remedy for several other ailments such as coughs, colds, fever, backache, toothache, sleeping sickness, venereal disease, malaria, inflammation, rheumatism, snakebite, tuberculosis, ulcers and pneumonia (Watt and Breyer-Brandwijk, 1962; Neuwinger, 1964; Galeffi et al., 1990; Rakuambo et al., 2004). This has earned the plant a popular vernacular name in the Hausa speaking north-western part of Nigeria "Uwar magnum guna", meaning mother of all medicines (Adebiyia et al., 2006). A short review about the secondary metabolites isolated from *Securidaca longepedunculata* is given below.

From the root bark of *Securidaca longepedunculata* Prista and Correira (1958) isolated as one of the major components, methyl salicylate. According to Moës (1966), the plant contains the same sapogenin, senegin, which is found in the folk medicinal plant species, *Polygala senega*. Delaude (1971) also isolated senegenin and senegenic acid from both *Securidaca longepedunculata* and *Polygala senega* as well as the sugars glucose, fructose, rhamnose, galactose and arabinose. Dugan et al. (1964) were able to obtain the aglycone, presenegenin; they showed it to be a quite normal triterpernoid, 2β,3β,27-trihydroxyolean-12-ene-23,28-dicarboxylic acid. On treatment with ethanolic HCl it is converted quantitavely to a mixture of senegenin and senegenic acid (polygalic acid). The new xanthone 1,7-dimethoxy-2-hydroxyxanthone was isolated by Galeffi et al. (1990) from the roots collected in Malawi. Surprisingly, Costa et al. (1992) reported the existence of ergot alkaloids as psychoactive compounds in the methanolic extract of the roots. Elymoclavine and dehydroelymoclavine were identified along with a novel ergoline alkaloid A. The presence of other alkaloids with an ergoline skeleton was confirmed (Scandola et al., 1994). Ergoline alkaloids are rarely found in higher plants. These alkaloids have previously mainly been isolated from fungi, and the question could be asked if they were not perhaps isolated from fungi growing on the bark of the *Securidaca* species? Mahmood et al. (1993) extracted 3,4,5-tri-*o*-caffeoylquinic acid, 4,5-di-*o*-caffeic acid and sinapic acid from the roots and demonstrated that 3,4,5-tri-*o*-caffeoylquinic acid has anti-HIV activity. Meli Lannang et al. (2006) recently isolated securidacaxanthone A, a heptaoxygenated xanthone from *Securidaca longepedunculata*.

From the stem bark, Kogan et al. (1968) isolated an alkaloid, which proved to be the very toxic securinine, previously extracted from the Euphorbiaceae species, *Securinega suffruticosa*. This remarkable alkaloid is a tricyclic decahydro-1,10-methano-pyrido (1,2-a)-azepin system fused with an α,β-unsaturated butenolide ring which is common in cardenolides (Horii et al., 1967). Biogenetically, it is derived from the amino acids tyrosine and lysine (piperidine ring). It is the first alkaloid derived from lysine with the N part of the two rings (Sankawa et al., 1974).

From the seeds, Smith et al. (1979) showed that the seed oil is a rich source of fatty acids (conjugated hydroxy dieonic fatty acids) and tricylglycerols (acetotriacylglycerols) of unusual structure.

From the leaves, Odebiyi (1978) reported that they are very rich in saponines. Kamwendo et al. (1985) screened the leaves in Malawi for phytochemical constituents and showed the presence of saponines, tannins, anthroquinones, sterols and terpenes; alkaloids and flavonoids were absent.

We report here on the isolation of two novel xanthones from *Securidaca longepedunculata* and their effect on rabbit corpus cavernosum smooth muscle.

2. Materials and methods

2.1. Plant material

The root bark of *Securidaca longepedunculata* was collected in Venda (South Africa) and a voucher specimen (NCR. 16) was deposited and identified at the H.G.W.J. Schweickerdt Herbarium (PRU), University of Pretoria.

2.2. Extraction and purification of xanthones

Dry root bark (634 g) of *Securidaca longepedunculata* was extracted in acetone by homogenizing the plant material using a blender and leaving it in a dark at room temperature for 8 days. The extraction process was repeated two times. The extract was filtered and concentrated to dryness under reduced pressure at 37 °C, yielding 11.0 g. The total extract was subjected to column chromatography using silica gel (Merck Kieselgel 60 (0.063–0.200 mm), 500 g), and eluted with the following solvent ratios of *n*-hexane:ethyl acetate: 100:0, 80:20, 60:40, 40:60, 20:80, 0:100, then with 10:90 methanol:ethyl acetate and finally with 100% methanol. The fractions (46) were collected and combined to seven sub-fractions based on the TLC results. All fractions were analysed for bioactivity as described below. The results showed that fraction 3 was the most active fraction and re-chromatographed using a silica gel column. The column was eluded with 80:20 *n*-hexane:ethyl acetate. Six fractions were obtained and after the bioassays showed that fraction 4 was the most active, it was further purified on a Sephadex LH-20 column. The column was packed with about 15 g of Sephadex LH-20 and fraction 4 applied (88 mg) dissolved in methanol. The column was eluated with methanol (100%) and 6 fractions were obtained. Fractions 3 (23 mg) and 6 (21 mg) were shown to contain pure compounds by means of TLC (Merck, Kieselgel 60 F_{254}) in different solvent systems and finally by ^{1}H NMR. These compounds were further analysed by different physicochemical methods to establish their chemical structure.

The general experimental procedures for the structure elucidation were as follows: melting points are uncorrected. IR spectra were recorded on a PerkinElmer 1310 spectrophotometer. UV spectra were recorded using a Pharmacia LKB-ultraspec 111 UV spectrophotometer. NMR spectra were recorded using a Bruker Avance DRX 500 MHz. Mass spectra were obtained with a JEOL JMS-AX505 W mass spectrometer.

2.3. Preparation of corpus cavernosal smooth muscle

Ethical clearance from the University of Pretoria's ethical committee was obtained for the following experiment: the penes of New Zealand rabbits (mass 2.0–2.3 kg) was obtained and kept in Krebs phosphate-buffered saline aerated with 95.0% O_2 and 5.0% CO_2 for less than 30 min. One or two strips of the corpus cavernosum smooth muscle (12 mm long and 1–2 mm thick) were dissected from each penis. The strips were mounted in an organ-bath chamber containing Krebs solution (pH 7.3) with the following composition: NaCl = 7.01 g/l, KCl = 0.34 g/l, KH_2PO_4 = 0.1 g/l, $NaHCO_3$ = 1.99 g/l, $CaCl_2$ = 0.2 g/l, $MgSO_4$ = 0.3 g/l and glucose = 1.8 g/l.

2.4. Smooth muscle relaxation bioassay

Strips of rabbit corpus cavernosum smooth muscle were mounted in a perfusion bath, with one end tied to the inside bottom of the perfusion bath and the other end to a thin wire connected to a Harvard isotonic force transducer for tension mea-

Table 1
^{1}H NMR and ^{13}C NMR chemical shifts of 1,3,6,8-tetrahydroxy-2,5-dimethoxyxanthone (**1**) and 1,6,8-trihydroxy-2,3,4,7-tetramethoxyxanthone (**2**) (δ, ppm, in $CDCl_3$)

Carbon no.	Compound **1** ^{13}C	^{1}H, mult, *J*	Compound **2** ^{13}C	^{1}H, mult, *J*
1	149.3		150.0	
2	127.5		132.8	
3	157.7		154.0	
4	93.1	6.25 s	135.6	
4a	153.0		149.8	
4b	148.8		157.9	
5	130.5		98.9	6.36 s
6	158.3		159.8	
7	98.3	6.45 s	128.3	
8	152.7		145.6	
8a	101.5		100.9	
8b	101.3		100.4	
9	183.7		183.7	
OMe	61.5	3.84 s	61.8	3.99 s
	60.7	3.88 s	61.6	4.14 s
			61.2	3.97 s
			61.1	3.92 s
OH		12.10 s		11.88
		11.80 s		11.38

surements (Drewes et al., 2002). Changes in isotonic tension were recorded on a chart polygraph by a recorder. The corpus cavernosum muscle was perfused with 2.0 ml Krebs PSS-buffered saline and oxygenated with 95% O_2 and 5% CO_2 for 5 min to ascertain a stable baseline recording. This was followed by perfusion with 2.0 ml of $CaCl_2$–PSS (17.8 mg/ml) to achieve muscle contraction. Baseline tension was set at the point of maximal contraction following the addition of calcium chloride into the experimental bath. The compounds to be analysed were dissolved in DMSO and added after a stable contraction baseline was obtained. The final compound concentration in a temperature controlled perfusion bath (37 °C) was 1.8×10^{-5} mg/ml. The same procedure (and concentration) was repeated for the positive control, Viagra (Pfizer). In these experiments the stimulation frequency used for rabbit strips was 9.0 Hz. Three replicates were used per treatment.

3. Results and discussion

Column chromatography of the acetone extract of the fresh root bark of *Securidaca longepedunculata* yield two pure novel xanthones, 1,3,6,8-tetrahydroxy-2,5-dimethoxyxanthone and 1,6,8-trihydroxy-2,3,4,7-tetramethoxyxanthone.

1,3,6,8-Tetrahydroxy-2,5-dimethoxyxanthone was isolated as yellow crystals with a melting point of 152–155 °C. The molecular formula $C_{15}H_{12}O_8$ was deduced from the low-resolution EIMS (M+ 310.1), ^{13}C, DEPT-135, and HMQC data. Its IR spectrum showed the presence of hydroxyl (3245 cm^{-1}) and carbonyl (1649 cm^{-1}) groups. The UV analysis was indicative for a xanthone skeleton with the presence of pre-hydroxy group(s) at positions 1 and/or 8. This was confirmed by ^{1}H NMR (Table 1) which showed two –OH signals at 12.10 and 11.80. The NMR spectra showed also two proton singlet signals at 6.25 and 6.45 (δ_C 93.1 and 98.3, respectively) and two methoxyl signals at 3.84 and 3.88 (δ_C 61.5 and 60.7). The above data suggested a compound with the structure depicted in Fig. 1, the HMBC spectra confirmed the given structure which showed correlations $H_{6.25}$/C-2, C-3, C-8a; $H_{6.41}$/C-4b, C-8, C-5, C-8b; $OMe_{3.84}$/C-2, $OMe_{3.88}$/C-5. The full characteristics of it are yellow crystals, mp 152–155 °C. UV (MeOH) λ_{max} nm: 237, 258, 330; NaOAc: 270, 355. (KBr), ν_{max} = 3245, 1649, 1599, 1512, 1278 1200, 1072 cm^{-1}; LREIMS (*m*/*z*), 310.1 [M^+]. ^{1}H NMR (500 MHz, $CDCl_3$): 12.1, 11.8 (s each, 2× OH), 6.25(s, H-4), 6.45 (s, H-7), 3.84, 3.88 (s each, 2× OMe). ^{13}C NMR (125 MHz, $CDCl_3$): δ183.7 (C-9), 158.3 (C-6), 157.7 (C-3), 153.0 (C-4a), 152.7 (C-8), 149.3(C-1), 148.8 (C-4b), 130.5 (C-5), 127.5 (C-2), 101.5(C-8a), 101.3 (C-8b), 93.1 (C-4), 98.3 (C-7), 61.5(C-OMe), 60.7(C-OMe).

1,6,8-Trihydroxy-2,3,4,7-tetramethoxyxanthone was obtained as pale yellow crystals with the molecular formula $C_{17}H_{16}O_9$ deduced from LREIMS and NMR spectroscopic analyses. Its IR spectrum showed the presence of hydroxyl and carbonyl groups. The absorption maxima at 265, 355 and 235 nm in the UV spectrum of it indicated a xanthone skeleton. The ^{13}C NMR spectrum (Table 1) displayed 17 carbon signals and separated by DEPT spectrum into Four methoxyl, a methine and twelve quaternary carbons (one for a carbonyl carbon). The ^{1}H NMR spectrum showed two singlet hydroxyl proton signals at δ 11.38, 11.88, an aromatic proton resonance at δ 6.36 (1H, s), and four singlets at δ 4.14, 3.99, 3.97 and 3.92 (3H each, δ_C 61.1, 61.8, 61.2 and 61.6 respectively) due to four methoxyl groups. From data above, the structure of it could be deduced to be a xanthone substituted by three hydroxyl

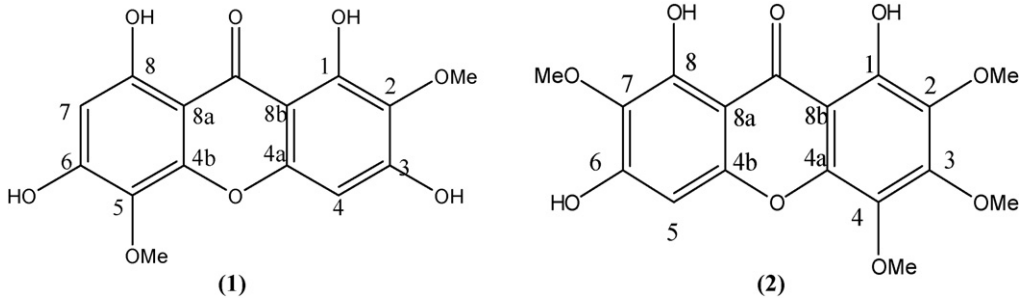

Fig. 1. Two novel xanthones, 1,3,6,8-tetrahydroxy-2,5-dimethoxyxanthone (**1**) and 1,6,8-trihydroxy-2,3,4,7-tetramethoxyxanthone (**2**) isolated from *Securidaca longepedunculata*.

Table 2
% Relaxation of pre-contracted rabbit corpus carvenosal smooth muscle in the presence of xanthones isolated from *Securidaca longepedunculata*

Compound	Average % relaxation (std)
1,3,6,8-Tetrahydroxy-2,5-dimethoxyxanthone	97.0 (5.7)
1,6,8-Trihydroxy-2,3,4,7-tetramethoxyxanthone	30.5 (4.8)
Viagra	100.0 (0.0)

All compounds were tested at 1.8×10^{-5} mg/ml. The DMSO solvent contracted the muscles 20.0% at a 1.0% concentration.

and four methoxyl functions. The positions of these substitution functions were further established according to its HMBC spectrum which showed correlation signals between H-5 and C-6, C-5, C-4b, C-8a; 2-OMe/C-2; 3-OMe/C-3; 4-OMe/C-4, and 7-OMe/C-7 respectively. Therefore, the structure of it was deduced to be 1,6,8-trihydroxy-2,3,4,7-tetramethoxyxanthone. Its full characteristics are pale yellow crystals, mp 201–204 °C. UV (MeOH) λ_{max} nm, 235, 265, 355; NaOAc, 265, 350. (KBr), ν_{max} = 3255, 1653, 1595, 1510, 1278, 1200, 1070 cm^{-1}; LREIMS (m/z), 364.08 [M^+]. ^{1}H NMR (500 MHz, $CDCl_3$): 11.38, 11.88 (s each, 2× OH), 6.36 (s, H-5), 4.14, 3.99, 3.97, 3.92 (s each, 4× OMe); ^{13}C NMR (125 MHz, $CDCl_3$): δ 183.7 (C-9), 159.8 (C-6), 157.9 (C-4b), 154.0 (C-3), 150.0 (C-1), 149.8 (C-4a), 145.6 (C-8), 135.6 (C-4), 132.8 (C-2), 128.3 (C-7), 100.9 (C-8a), 100.4 (C-8b), 98.9 (C-5), 61.8, 61.6, 61.2, 61.1 (4× –OMe).

Securidaca longepedunculata's xanthones stimulated the relaxation of corpus cavenosum smooth muscle (Table 2) between 0 and 60 s after the application of the compounds in a frequency-dependent manner. At a concentration of 1.8×10^{-5} mg/ml, 1,3,6,8-tetrahydroxy-2,5-dimethoxyxanthone relaxed the muscles 97% whereas 1,6,8-trihydroxy-2,3,4,7-tetramethoxyxanthone relaxed them only 30.5%. The positive control, Viagra relaxed the muscles 100.0% at this concentration. It was also found that the 1.0% of DMSO (negative control) used to dissolve the compounds, contracted the muscles by 20.0%.

"The erect penis has always been a symbol of power, virility and fertility" (Guirguis, 1998). In response to recent advances in synthetic medication, there has been a renewed interest in medicinal plants in the treatment of sexual dysfunction (Adimoelja, 2000). Several medicinal plants are used in South Africa to treat erectile dysfunction (Rakuambo et al., 2006). Erection cannot take place if the corpus carvenosum smooth muscle is contracted. The contraction of the corpus carvenosum smooth muscle is mediated by both translocations of calcium from extracellular sources and the release from intracellular sites especially the sarcoplasmic reticulum (Levin et al., 1997). Although there is a strong relationship between relaxation of the corpus carvenosum smooth muscle and sexual function (Keaton and Clark, 1997), it is also well known that there are many other factors that contribute to the deterioration of sexual function (Colabro et al., 1996).

Previous in vitro bioassays of *Securidaca longepedunculata* (Rakuambo et al., 2006) showed that its extract has potential as a future candidate for the treatment of erectile dysfunction. The Vhavenda people usually dissolve its powdered root bark in carefully measured portions with Mabundu (a traditional drink from water and different grains) and consume it immediately or within 2 days. It is known that the species is poisonous probably because it contains securinine as well as other alkaloids (Van Wyk et al., 1997).

These preliminary results can be seen as the groundwork for further studies on toxicity and in vivo activity and mechanism of action experiments. According to Drewes et al. (2003) the search is still on for a natural product that can deal successfully with erectile dysfunction.

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