

Full Length Research Paper

A new pterocarpan from the leaves of *Abrus precatorius* L.

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A pterocarpan, 2,3,4,8-tetramethoxy-6a,11a-dihydro-6H-benzo[4,5]furo[3,2-c]chromene-7,9-diol (compound 1) was isolated from the dichloromethane extract of the leaves of *Abrus precatorius* L. The structure of the compound was elucidated by detailed spectroscopic analysis such as ¹H NMR, ¹³C NMR, distortionless enhancement by polarisation transfer (DEPT), heteronuclear multiple-quantum correlation (HMQC) and heteronuclear multiple-bond correlation (HMBC). The crude extracts displayed brine shrimp lethality and *in vitro* antimalarial activity. The methanolic crude extract demonstrated an A/B ratio of 9.7 signifying that it is more toxic against *Plasmodium falciparum* than brine shrimp.

Key words: Fabaceae, Papilionaceae, *Abrus precatorius*, pterocarpan, brine shrimp lethality, antiplasmodial activity.

INTRODUCTION

The plant *Abrus precatorius* L. (family: Fabaceae, sub-family: Papilionaceae) is a vine originally native to India that is now commonly distributed throughout tropical and subtropical regions of the world (Choi et al., 1989). Poisoning by the seeds of these species has been well documented (Morton, 1977, 1995; Anonymous, 1969; Saganuwan et al., 2011) with abrin, the glycoprotein responsible, being regarded as one of the most potent of all known toxins (Olsnes et al., 1974). Despite the known toxicity of *A. precatorius* seeds, its roots have been used since early 19th century as a substitute for licorice root, the source of the sweet oleanane-type triterpene glycoside, glycyrrhizin (Dymock et al., 1893; Hooper,

1894; Uphof, 2001; Prathyusha et al., 2010; Pokharkar et al., 2011; Attal et al., 2010). In *in vivo* studies, different organic extracts of the seeds induced antifertility activity (Abu et al., 2012), analgesic activity (Monago and Alumanah, 2005), antimicrobial activity (Bobbarala and Vadlapudi, 2009), hepatoprotective activity (Battu and Kumar, 2009), and can protect the kidney against alcohol-induced parenchymal injury (Ligha et al., 2009), while that of leaves induced analgesic activity (Nagaveni et al., 2012) and may be used in the management of asthma (Taur and Patil, 2012). The root decoction is used against vomiting, dysentery, uterine prolapse, epilepsy, stomachache, convulsions in children, conjunctivitis,

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asthma and as an aphrodisiac (Sujit, 2011; Janakiraman et al., 2012). The dried powdered leaves are crushed and the juice drunk against cough, fever and dizziness (Chhabra et al., 1990). The chemical composition (Mollik et al., 2009; Rajaram and Janardhanan, 1992) and nutritional potential (Rajaram and Janardhanan, 1992) of the seeds were investigated. The present study reports the isolation and structure elucidation by various spectroscopic techniques of a new pterocarpan, 2,3,4,8-tetramethoxy-6a,11a-dihydro-6H-benzo[4,5]furo[3,2c]chromene-7,9-diol (compound 1) from the dichloromethane extract of the leaves of *A. precatorius*. The crude extracts were evaluated for brine shrimp lethality and *in vitro* antiplasmodial activity.

MATERIALS AND METHODS

General experimental procedures

The melting point of the pure compound was determined on a Gallenkamp melting point apparatus (Sanyo, United Kingdom) with open capillary tubes and was uncorrected. The NMR spectra were obtained from Varian Gemini 200 MHz and a Bruker Avance DPX 300 MHz machine. MS analysis was performed on pure solid samples using the direct insertion probe (DIP) on a Fission Platform Mass Spectrometer operated at 70 eV and mass range set at 38-400 a.m.u. and using Atmospheric Pressure Chemical Ionization (APCI) on a Finnigan LCQ Deca machine, in either the positive mode (gives M+H) or negative mode (gives M-H as quasi molecular ion). Pre-coated plastic sheets (Polygram[®] sil G/UV₂₅₄) and aluminium sheets (Alugram[®] sil G/UV₂₅₄ of 20 cm by 20 cm Machery-Nagel GmbH and Co., KG) were used for Thin Layer Chromatography (TLC). Column chromatography was carried out using slurry packing with Kiesegel silica gel (0.063-0.2/70-230 mesh ASTM Kobian Kenya Ltd., Nairobi).

Plant

The leaves of the plant *A. precatorius* L. were collected in October 2000 based on information given by various herbalists on its use for the treatment of various diseases specifically malaria from the Mombasa County (latitude -4.05°, longitude 39.67°), Kenya. It was identified by Mr. Simon Mathenge, a taxonomist at the Botany Department, University of Nairobi and a voucher specimen (CNM/SM/09/01) deposited at the Nairobi University herbarium. The plant material was dried under shade and ground using a motor grinding machine.

Extraction and isolation

The air dried powdered leaves of *A. precatorius* (600 g) were extracted by maceration successively in hexane, dichloromethane and methanol (each 1200 ml, 3×48 h). The combined extracts of each solvent were concentrated under reduced pressure below 50°C to get the respective crude extracts. Dichloromethane crude extract of *A. precatorius* (2.98 g) was chromatographed over silica gel column (30 g, 100-200 mesh), eluted with gradients of EtOAc in *n*-hexane and gave 17 fractions (300 ml each). Fractions (14 to 17) obtained on elution with 100% EtOAc yielded compound 1 on repeated washing with diethyl ether and crystallization from MeOH with an *R_f* of value 0.70 (*n*-hexane-EtOAc, 50:50).

2,3,4,8-Tetramethoxy-6a,11a-dihydro-6H-benzo[4,5]furo[3,2-c]chromene-7,9-diol (compound 1)

Yield 25 mg (white crystals). m.p. found: 175.7-176.2°C; IR, 3400, 3299, 2900, 1576.2, 1306.7, 918.1, 872.7, 838.0, 730.0, 774.4 cm⁻¹; ¹H NMR spectral data (300 MHz, CDCl₃): δ 6.65 (1H, s, H-1), 6.61 (1H, s, H-10), 4.34 (1H, dd, J = 4.8, 10.8 Hz, H-6α), 3.55 (1H, m, H-6a), 3.68 (1H, t, J = 10.8, 10.9 Hz, H-6β), 5.41 (1H, s, 9-OH), 5.46 (1H, d, J = 6.9 Hz, H-11a), 5.63 (1H, s, 7-OH), 3.92 (3H, s, 4-OMe), 3.93 (3H, s, 3-OMe), 3.88 (3H, s, 2-OMe), 3.99 (3H, s, 8-OMe); ¹³C NMR Spectral data (75 MHz, CDCl₃): δ 148.1 (C-2), 144.2 (C-9), 144.1 (C-10a), 139.3 (C-8), 138.8 (C-7), 138.5 (C-4a), 138.1 (C-3), 137.3 (C-4), 123.0 (C-7a), 115.1 (C-1a), 105.1 (C-10), 104.1 (C-1), 78.5 (C-11a), 67.0 (C-6), 61.7 (8-OMe), 61.4 (4-OMe), 60.7 (3-OMe), 56.7 (2-OMe), 41.3 (C-6a); APCI (+ve mode, m/z (rel. int. %): 376 (88.6), 362 (20), 361 (100), 315 (9.3), 194 (31.4), 183 (12.1%), 181 (35), 179(36.4), 173 (18.9), 151 (10), 136 (21.4), 131 (16.4), 123 (14.3), 115 (30), 102 (17.9), 94 (12.1), 91 (27.1), 89 (19.3), 81 (11.4), 77 (27.1), 69(23.6), 65 (27.1), 63 (12.9), 53 (22.1), 51 (17.1), 43 (14.3), 39 (19.3).

Toxicity testing against the brine shrimp

Hatching shrimp

Brine shrimp eggs, *Artemia salina* Leach were hatched in artificial seawater prepared by dissolving 38 g of sea salt (Sigma chemicals Co., UK) in 1 L of distilled water. After 48 h incubation at room temperature (22 to 29°C), the larvae (nauplii) were attracted to one side of the vessel with a light source and collected with pipette. Nauplii were separated from eggs by aliquoting them three times in small beakers containing seawater.

Brine shrimp bioassay

The bioactivity of the extracts was monitored by the brine shrimp lethality test (Wanyoike et al., 2004). Samples were dissolved in dimethylsulphoxide (DMSO) and diluted with artificial sea salt water so that final concentration of DMSO did not exceed 0.05%. Fifty microliters of sea salt water was placed in all the wells of the 96-well microtiter plate. Fifty microliters of 4000 ppm of the plant extract was placed in row one and a two-fold dilution carried out down the column. The last row was left with sea salt water and DMSO only served as the drug free control. 100 µl of suspension of nauplii containing about 10 larvae was added into each well and incubated for 24 h. The plates were then examined under a microscope (12.5×) and the number of dead nauplii in each well was counted. 100 µl of methanol was then added and after 10 min, the total numbers of shrimp in each well were counted and recorded. Lethality concentration fifties (LC₅₀ values) for each assay were calculated by taking average of three experiments using a Finney Probit analysis program on an IBM computer (Wanyoike et al., 2004).

In vitro antimalarial test

Cultures of *P. falciparum*

Laboratory adapted *P. falciparum* cultures of the international reference isolates VI/S (chloroquine resistant) were used. The strains have been cultured and maintained at the Faculty of Pharmacy, University of Nairobi, Nairobi. The culture medium was a variation of that described by Wanyoike (2004) and consisted of

Table 1. Percent yield of extracts, brine shrimp toxicity and *in vitro* antiplasmodial activity of the extracts of the leaves of *A. precatorius*.

Extract	Hexane	Dichloromethane	Methanol	Emetine hydrochloride*	Chloroquine*
Percent yield of extract	0.70	0.67	1.00		
Brine shrimp toxicity: LC ₅₀ ±SD (µg/ml)	927.8±2.2	>1000.0	415.3±1.4	20.1±0.2	-
<i>In vitro</i> antiplasmodial activity: IC ₅₀ ±SD (µg/ml)	-	-	43.0±0.9	-	0.105±0.0

*Included as a positive control.

RPMI 1640 supplemented with 10% human serum, 25 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid and 25 mM NaHCO₃. Human type O+ erythrocytes (<28 days old) served as host cells and the cultures were incubated at 37°C in an atmosphere of 3% CO₂, 5% O₂ and 92% N₂.

Antiplasmodial bioassay

The *in vitro* semi-automated microdilution assay technique that measured the ability of the extracts to inhibit the incorporation of [³H]hypoxanthine into the malaria parasite was used (Wanyoike et al., 2004). For the test, 25 µl aliquots of culture medium were added to all the wells of a 96 well flat-bottom microculture plate (Costar Glass Works, Cambridge, UK). Aliquots (25 µl) of the test solutions were added, in duplicate, to the first wells, and a Titertek motorized hand diluter (Flow Laboratories, Uxbridge, UK) was used to make serial 2-fold dilutions of each sample over a 64-fold concentration range. Aliquots (200 µl) of a 1.5% v/v suspension of parasitized erythrocytes in culture medium (0.4% parasitemia; growth rate > 3-fold per 48 h) were added to all test wells. Parasitized and non-parasitized erythrocytes were incorporated into all tests. The plates were incubated at 37°C in a gas mixture 3% CO₂, 5% O₂ and 92% N₂. After 48 h, each well was pulsed with 25 µl of culture medium containing 0.5 µCi of [³H] hypoxanthine and the plates were incubated for a further 18 h. The contents of each well were then harvested onto glass fiber filters, washed thoroughly with distilled water, dried and the radioactivity in counts per minute (cpm) measured by liquid scintillation.

Computation of the concentration of drug causing 50% inhibition of [³H]hypoxanthine uptake (IC₅₀) was carried out by interpolation after logarithmic transformation of both concentration and cpm values using the formula:

$$IC_{50} = \text{antilog} \left[\frac{(\log X_1 + [(\log Y_{50} - \log Y_1) \times (\log X_2 - \log X_1)])}{\log Y_2 - \log Y_1} \right]$$

where Y₅₀ is the cpm value midway between parasitized and non-parasitized control cultures and X₁, Y₁, X₂ and Y₂ are the concentrations and cpm values for the data points above and below the cpm midpoints (Wanyoike et al., 2004).

RESULTS AND DISCUSSION

The leaves of the plant *A. precatorius* were successively extracted with hexane, dichloromethane and methanol and the percent yield of each extract is as shown in Table 1. The brine shrimp toxicity and *in vitro* antiplasmodial

activity of the extracts of the leaves are also shown in Table 1.

Brine shrimp lethality and *in vitro* antiplasmodial test

The crude extracts (hexane, dichloromethane and methanol) of the leaves of *A. precatorius* plant were subjected to brine shrimp lethality test and their LC₅₀ values were determined. The *in vitro* antimalarial activities of the methanolic crude extract against chloroquine resistant VI/S strain of *P. falciparum* were also investigated and its IC₅₀ value determined. The methanolic crude extract of *A. precatorius* displayed a higher activity against brine shrimp (415.3 µg/ml). The hexane (927.8 µg/ml) and dichloromethane (>1000.00 µg/ml) extracts were about 2 times less active than the methanolic extract. The methanolic extract displayed an *in vitro* antiplasmodial activity of 43.0 µg/ml.

A/B ratio

When searching for new antimalarial agents, it is important to distinguish between specificity of action and non-selective toxicity. An estimation of therapeutic index in which the desired biological activity is compared with general toxicity is one in which selectivity of activity may be assessed (Abu et al., 2012). The brine shrimp lethality and activity against *P. falciparum* has been compared for the extracts and a ratio A/B for the two calculated (Monago and Alumanah, 2005). A value greater than one is considered indicative of more selective activity against *P. falciparum*, and a value less than one of more selectivity to brine shrimp.

The activities of the methanolic crude extract of *A. precatorius* against brine shrimp (A µg/ml) and *P. falciparum* (B µg/ml) *in vitro* was calculated to be 9.7. The A/B ratio of the methanolic extract of *A. precatorius* (9.7) demonstrates that it is more toxic against *P. falciparum* than brine shrimp.

Structure elucidation

Compound 1 was isolated from the dichloromethane

Table 2. ^1H and ^{13}C NMR data for compound 1.

Carbon	$\delta_{\text{C}}^{\text{c,d}}$	$\delta_{\text{H}}^{\text{b}}$	HMQC	HMBC
1	104.1 (d)	6.65 (1H, s)	6.65	C-11a
1a	115.1 (s)	-	-	C-6a
2	148.1 (s)	-	-	2-OMe
3	138.1 (s)	-	-	C-1
4	137.3 (s)	-	-	4-OMe
4a	138.5 (s)	-	-	C-1, C-11a, C-6
6	67.0 (t)	3.68 (1H, t, 10.8, 10.9) 4.34 (1H, dd, 4.8, 10.8)	3.68 4.34	C-11a
6a	41.3 (d)	3.55 (1H, m)	3.55	-
7a	123.0 (s)	-	-	7-OH, C-6, C-10, C-11a
7	138.8 (s)	5.63 (1H, s)	-	C-6a
8	139.3 (s)	-	-	8-OMe, 7-OH, 9-OH
9	144.2 (s)	5.41 (1H, s)	-	-
10	105.1 (d)	6.61 (1H, s)	6.61	9-OH
10a	144.1 (s)	-	-	C-6a, C-7, C-11a
11a	78.5 (d)	5.46 (1H, d, 6.9)	5.46	C-6, C-1
2-OMe	56.7 (q)	3.88 (3H, s)	3.88	-
3-OMe	60.7 (q)	3.93 (3H, s)	3.93	-
4-OMe	61.4 (q)	3.92 (3H, s)	3.92	-
8-OMe	61.7 (q)	3.99 (3H, s)	3.99	-

extract of the leaves of *A. precatorius* as white crystals. The IR spectrum showed peaks at 3299 and 3400 cm^{-1} (strong, broad hydroxyl groups), together with at 1307 cm^{-1} (C-O stretching), 2900 cm^{-1} (C-H stretching) and 1576 cm^{-1} (aromatic C=C stretching).

The ^1H NMR spectral data of compound 1 (Table 2) suggested a pterocarpan structure due to the splitting pattern of the protons at 5.46 (*d*, $J = 6.9$ Hz, H-11a), 4.34 (*dd*, $J = 4.8, 10.8$ Hz, H-6 α), 3.55 (*m*, H-6a) and 3.68 (*t*, $J = 10.8, 10.9$ Hz, H-6 β), related to the protons of the hetrocyclic ring B and the bridging protons of B and C rings, respectively (Tarus et al., 2002; Prathyusha et al., 2010). The chemical shifts appearing at δ 6.65 and 6.61 were assigned to H-1 and H-10, respectively.

In addition, compound 1 possessed four methoxy groups, which appeared at δ 3.92 (3H, s, 4-OMe), 3.93 (3H, s, 3-OMe), 3.88 (3H, s, 2-OMe) and 3.99 (3H, s, 8-OMe). These were placed on rings A and D. Two hydroxyl groups appearing at δ 5.41 (s, 9-OH) and 5.63 (s, 7-OH) were assigned to the two OH groups attached to carbon 9 and 7, respectively.

A total of 19 signals were observed on ^{13}C NMR spectrum, while the Distortionless Enhancement by Polarisation Transfer (DEPT) showed a total of 1 methylene, 4 methyl and 4 methine groups (Table 2). Two methine protons were observed at the chiral centres

δ 5.46 (1H, d, $J = 6.9$ Hz, H-11a) and at δ 3.55 (1H, m, H-6a). The ^1H - ^1H COSY NMR spectrum showed coupling between protons δ 5.46 (H-11a) and δ 3.55 (H-6a). It also showed coupling between protons δ 4.34 (H-6 α) and δ 3.68 (H-6 β), δ 4.34 (H-6 α) and δ 3.55 (H-6a), δ 5.46 (H-11a) and δ 3.55 (H-6a).

The Heteronuclear Multiple-Quantum Correlation (HMQC) spectrum for compound 1 confirmed that the proton at δ 6.65 (H-1) was attached to carbon at δ 104.1 (C-1) while that at δ 5.46 (H-11a) was attached to carbon at δ 78.5 (C-11a). The proton at δ 3.55 (H-6a) was attached to carbon at δ 41.3 (C-6a), while that at δ 3.68 (H-6 β) and 4.34 (H-6 α) were attached to carbon at δ 67.0 (C-6). The methoxylated protons at δ 3.88, 3.93, 3.92 and 3.99 were attached to carbons at 56.7 (C-2), 60.7 (C-3), 61.4 (C-4) and 61.7 (C-8), respectively. Heteronuclear Multiple-Bond Correlation (HMBC) assisted in the assignment of the quaternary carbons as shown in Table 2.

The mass spectrum of compound 1 showed a peak at m/z 376 (88.6%) corresponding to the chemical formula $\text{C}_{19}\text{H}_{20}\text{O}_8$. The suggested structure of compound 1 is 2,3,4,8-Tetramethoxy-6a,11a-dihydro-6H-benzo[4,5] furo [3,2-c]chromene-7,9-diol (Figure 1). It is a hitherto unknown natural product and has been isolated for the first time from the leaves of *A. precatorius*.

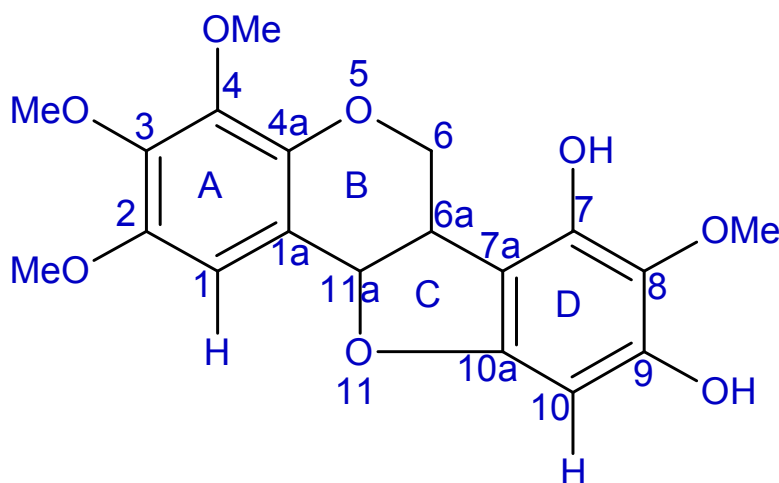


Figure 1. Structure of compound 1 2,3,4,8-Tetramethoxy-6a,11a-dihydro-6H-benzo[4,5]furo[3,2-c]chromene-7,9-diol.

Conclusion

A new pterocarpan (compound 1) from the dichloromethane extract of the leaves of *A. precatorius* has been isolated and reported for the first time. The methanol extract of the leaves showed *in vitro* antiplasmodial activity confirming the claim by the communities for the use of this plant in treating malaria.

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Conflict of interest

The authors declare that they have no conflict of interest.

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