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Short communication

Serotonin transporter affinity of (−)-loliolide, a monoterpene lactone from *Mondia whitei*

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Abstract

Mondia whitei (Apocynaceae) is used in traditional medicine to treat nervous disorders. Previous studies have shown *in vivo* antidepressant-like activity in the forced swimming test and affinity to the serotonin transporter of an ethanolic leaf extract of *M. whitei*. The aim of this study was to isolate the compound(s) responsible for *in-vitro* serotonin transporter affinity in *M. whitei*. Bioassay guided isolation lead to the identification of the monoterpene lactone (−)-loliolide. An ethanol extract was prepared from dry leaves. The residue was dissolved in ethyl acetate, extracted with water by liquid–liquid partitioning. This was followed by VLC fractionation. Through HPLC-UV separation the active compound was isolated and characterized by GC-MS, LC-MS and ^{1}H-NMR. The activity of (−)-loliolide was tested in a serotonin transporter binding assay using [^{3}H]-citalopram as ligand, giving an IC_{50}-value of 997 μM, corresponding to a K_i-value of 409 μM. Loliolide is a non-nitrogenous compound and might bind to the transporter in a different way to nitrogen-containing inhibitors. The results provide a rationale for the use of *M. whitei* in the treatment of depression and other central nervous system diseases in traditional medicine.

Keywords: [^{3}H]-citalopram; Depression; Loliolide; *Mondia whitei*; Radioligand binding assay; Serotonin transporter

1. Introduction

Depression is an ailment that is highly disabling. It is estimated that depression at one time or another will affect 25% of the world population (WHO, 2001). In southern Africa, a place traumatized by civil wars, political transition and AIDS, depressive disorders are common. The western health care system in southern Africa is overburdened, so patients are often relying on traditional medicine. However, African traditional healers do not recognize depression as an illness, though a number of other states and conditions with symptoms similar to depression are known, for example the condition 'being put down' by the ancestors (Stafford et al., 2008). Persons inflicted with curses and evil spirits often have symptoms that resemble a depressed state accompanied by lethargy. The incongruence between indigenous and western disease nomenclature makes it more difficult to evaluate the use of plants in a western scientific paradigm.

In a screening programme of plants used for depression-like disorders in traditional medicine in South Africa, an ethanolic extract of *Mondia whitei* leaves showed affinity to the serotonin transporter (SERT) (Nielsen et al., 2004). An ethanolic leaf extract of *M. whitei* was tested for affinity to the serotonin-, noradrenalin- and dopamine transporters in COS7 cells transfected with human transporters. The *M. whitei* extract had affinity to the serotonin transporter, but not to the noradrenaline or dopamine transporters (Pedersen et al., 2008). Further studies in rats showed that ethanolic extracts had activity in a forced swimming test indicating a possible antidepressant-like effect (Pedersen et al., 2008). These *in vivo* results also indicate that the active constituents are able to pass the blood brain barrier.

M. whitei is a robust forest climber, which has been used in African traditional medicine for centuries. Mainly the roots are used in medical treatment but the leaves and the entire plant has been utilized as well. The roots have a pleasant aromatic

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character and taste not unlike cinnamon and vanilla. Throughout Africa the dried roots are chewed and the sap swallowed as an aphrodisiac (Neuwinger, 2000). Root bark is used in Zimbabwe in a preparation known as *vuka-vuka* (Shona, which translates as wake-up, wake-up), which is reported to be a 'natural viagra', and is used as a male sexual stimulant (Hostettmann et al., 2000). In South Africa, the Zulu call it *uMondi* from which the genus gets its name. The roots are used in tonics, as an appetite stimulant, against abdominal pains, constipation and in the treatment of heartburn (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996). Root extracts have shown an androgenic effect in male rats (Watcho et al., 2004). Decoctions of the leaves are used as antispasmodics in pregnant women (Koorbanally et al., 2000). Water extracts yielded a very low toxicity (Watcho et al., 2004).

Despite its long and extensive use, there have been only a few studies on the isolation of bioactive compounds from *M. whitei*. A potent tyrosinase inhibitor, 2-hydroxy-4-methoxy-benzaldehyde, along with three chlorinated coumarinolignans have been isolated from the roots (Kubo and Kinst-Hori, 1999; Patnam et al., 2005).

Based on the *in vivo* activity and transporter selectivity, we decided to carry out a bioassay-guided isolation of the compound in *M. whitei* leaves with affinity to the SERT.

2. Materials and methods

2.1. Plant material

Leaves of *M. whitei* (Hook. f.) Skeels (Apocynaceae) were collected at the botanical garden at the University of KwaZulu-Natal, Pietermaritzburg (29°37′29S 30°24′15E Alt: 600 m) KwaZulu-Natal, South Africa. G. I. Stafford authenticated the plant material. A voucher specimen (*Stafford 43 NU*) was deposited in the Bews Herbarium (NU), University of KwaZulu-Natal, Pietermaritzburg.

2.2. Extraction

Fifty grams of ground dry *M. whitei* leaves were extracted with absolute ethanol (3 × 500 ml). During each extraction the plant material was sonicated for 60 min. The extract was evaporated to dryness *in vacuo* and the residue dissolved in 200 ml ethyl acetate. The solution was partitioned against 3 × 200 ml Milli-Q water. The ethyl acetate phase was evaporated to dryness (1.45 g).

2.3. Bioassay-guided fractionation and isolation

2.3.1. Vacuum liquid chromatography

The extract was dissolved in 100 ml ethyl acetate. To the solution was added 15 g celite and residual water was removed by co-evaporation with 50 ml heptane. The sample on celite was fractionated on Silicagel 60 (0.015–0.040 mm) on a 40 × 150 mm column. Two hundred ml of each of the following solvents were used as eluents: Toluene; toluene:ethyl acetate 80:20; 50:50; 20:80 (4 × 50 ml); ethyl acetate (4 × 50 ml); ethyl acetate:ethanol 80:20 (4 × 50 ml). Finally the column was eluted with 5 × 50 ml methanol. The fractions were tested in the SERT binding assay (Fig. 1). One of the active fractions from VLC (toluene:ethyl acetate 20:80) was further purified by preparative HPLC.

2.3.2. Preparative HPLC

Preparative HPLC was carried out on a Waters 1525 Binary HPLC Pump, connected to a Waters 2996 Photodiode Array Detector. A Luna-C_{18} column was used (250 × 21.2 mm, 5 µm, Phenomenex) eluted with a mixture of acetonitrile and Milli-Q water. The eluent composition was A: 100% Milli-Q water and B: 100% acetonitrile. The column was eluted with a linear gradient (A:B) from 100:0 (T_0) to 0:100 (T_{30}) to 0:100 (T_{60}) at a flow-rate of 10.0 ml/min. Samples (450 µl) were filtered through a 0.45 µM filter before each injection. Five fractions

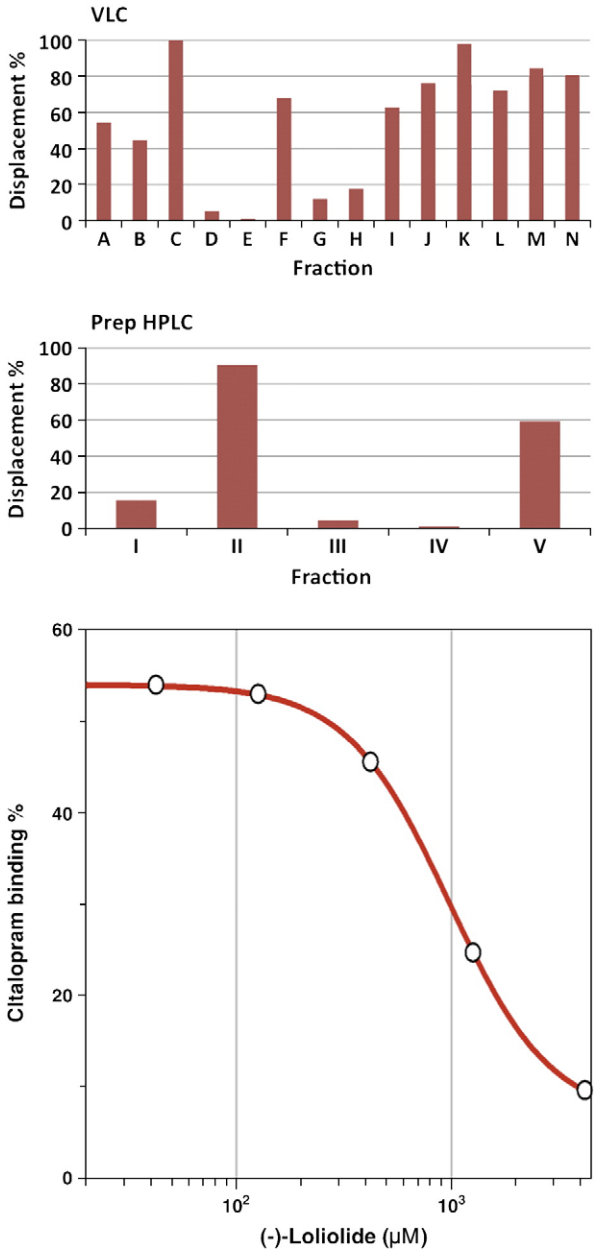

Fig. 1. Displacement of citalopram by VLC and HPLC fractions; and determination of IC_{50}-value for loliolide in the serotonin transporter assay.