Phytochemistry Letters 10 (2014) 101–106

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Artifactual generation of an alkaloid in the course of *Mondia whitei* (Hook.f.) Skeels roots extraction: A clue to endogenous-formed bioactive compounds?

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ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form 7 August 2014
Accepted 10 August 2014
Available online 23 August 2014

Keywords:
Mondia whitei (Hook.f.) Skeels
Apocynaceae
Artifact
Bioactivity
Neurotransmitters
Alkaloids

ABSTRACT

Introduction: The roots of *Mondia whitei* (Hook.f.) Skeels are widely used across Africa for the treatment of depression, asthenia and sexual disorders. The botanical family Apocynaceae infers the possible presence of alkaloids, which could be responsible for the reported biological properties.
Methods: Preparation of raw alkaloids extracts induced a reaction between a compound of the root and ammonia, yielding an alkaloid artifact. The precursor and reaction product were identified by HPLC–UV–ESI-MS. The precursor's reactivity toward neurotransmitters was investigated to highlight a possible mechanism of action of *M. whitei*.
Results: The compound 2-hydroxy-4-methoxybenzaldehyde was identified as the artifactual alkaloid precursor. Its isomers (vanillin and isovanillin) do not show detectable reactivity toward ammonia. Mass spectrometry and TLC analyses confirmed the reactivity of 2-hydroxy-4-methoxybenzaldehyde toward dopamine, γ-aminobutyric acid, norepinephrine and serotonin.
Conclusion: M. whitei contains a molecule, 2-hydroxy-4-methoxybenzaldehyde, able to generate alkaloid artifacts by reaction with the neurotransmitters dopamine, γ-aminobutyric acid, norepinephrine and serotonin; these stable derivatives might explain the root's alleged bioactivity.

1. Introduction

Mondia whitei (Hook.f.) Skeels is widely distributed across sub-Saharan Africa and its roots are used in folk medicine for a variety of diseases and conditions (Venter et al., 2009).

The remedy is presented as being able to treat asthma in children, cough and bronchitis, headache, depression, constipation, poor appetite, anorexia, abdominal and stomach pain, gastrointestinal disorders, vomiting, post-partum bleeding, gonorrhea, malaria, schistosomiasis and would also have an oxytocic activity (Aremu et al., 2011; Neuwinger, 2000; Pedersen et al., 2008). The roots are frequently chewed by men as a tonic and aphrodisiac, for the treatment of erectile dysfunction, sexual asthenia and to increase sperm production. A few studies aimed at unveiling the mechanism underlying the sexual dysfunction activity, but with conflicting results (Lampiao et al., 2008; Martey and He, 2010; Watcho et al., 2006; Watcho et al., 2001).

The root of *M. whitei* has been shown to harbor polyphenols (such as flavonoids and tannins) (Abdou Bouba et al., 2010; Pedersen et al., 2008), coumarinolignans (Patnam et al., 2005), aromatic volatile compounds such as 2-hydroxy-4-methoxybenzaldehyde and 3-hydroxy-4-methoxybenzaldehyde (isovanillin) (Mukonyi and Ndiege, 2001), and a glycoside of 2-hydroxy-4-methoxybenzaldehyde (Msonthi, 1991). So far, only two compounds present in *M. whitei* could be related to a bioactivity: *(i)* (−)-loliolide, a monoterpene lactone, found in the leaves for the treatment of depression (Neergaard et al., 2010) and *(ii)* 2-hydroxy-4-methoxybenzaldehyde for the inhibition of tyrosinase (Kubo and Kinst-Hori, 1999).

As *M. whitei* belongs to Apocynaceae, a family generally acknowledged for its content in alkaloids, several studies have

Abbreviations: 2H4MBZA, 2-hydroxy-4-methoxybenzaldehyde; ESI-MS, electrospray ionization-mass spectrometry; EtAc, ethyl acetate; EtOEt, diethyl ether; GABA, γ-aminobutyric acid; GC–MS, gas chromatography–mass spectrometry; MeOH, methanol; Rf, retention factor; R_t, retention time.

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http://dx.doi.org/10.1016/j.phytol.2014.08.012
1874-3900/

been performed for this class of compounds, but with conflicting results regarding their presence or absence (Ameyaw et al., 2009; Fred-Jaiyesimi and Ogunjobi, 2013; Kerharo, 1974; Pedersen et al., 2008).

The present aimed at isolating the eventual alkaloids of *M. whitei*'s root, and revealed that ammonia employed in the extraction process could generate an amine-bearing artifact. The precursor molecule was examined for its potential reactivity toward biogenic amines, what may explain the alleged activity of the root.

2. Results and discussion

2.1. M. whitei *roots treated with ammonia generate an alkaloid artifact*

Eight fractions (Alk1 to Alk4 and W1 to W4) of *M. whitei* roots were tested for their content in alkaloids by TLC with Dragendorff's reagent detection. From these fractions, only Alk2 displayed a potential content in alkaloids, as revealed by spots at retention factors (Rf) 0.52 (bright orange) and 0.90 (pale brown) (Fig. 1A).

As the Alk2 extraction relies on the use of ammonia via process B (powder moistening with 25% ammonia and extraction with dichloromethane overnight), the dry powder of *M. whitei* may contain a compound able to react with NH_3 during this moisturizing step to form an alkaloid artifact, subsequently extracted and detected.

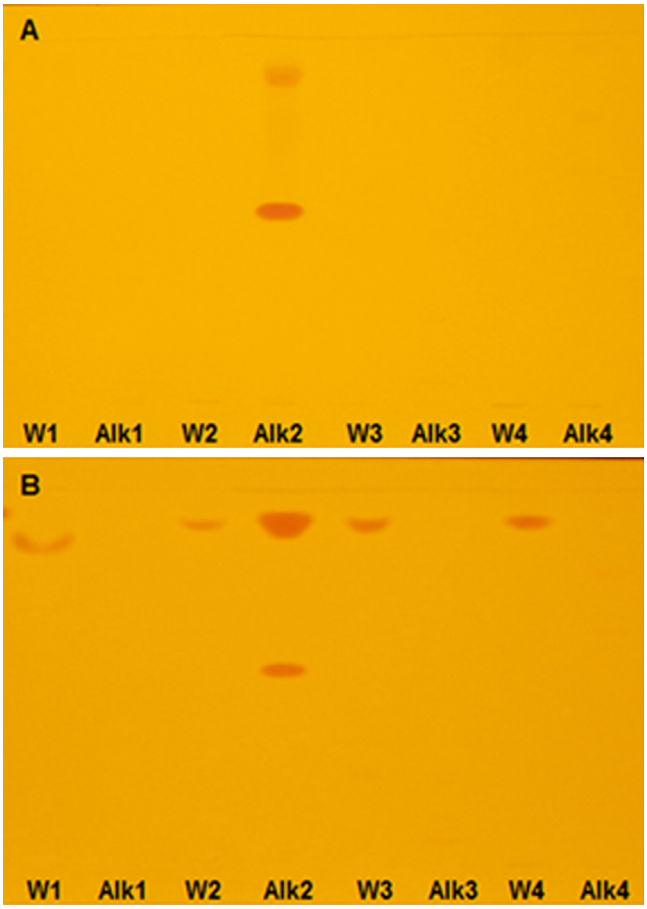

Fig. 1. TLC profiles obtained for total alkaloids extracts (Alk) and wash solutions (W): 10 μl of 10 mg/ml solution were deposited. Mobile phase was composed of EtAC/MeOH/H_2O (80:12:8). Plates were revealed with Dragendorff's spray reagent (A) or with Dragendorff's reagent following treatment in a NH_3-saturated environment before spraying (B).

To confirm this hypothesis, a second migration was performed using the same conditions, TLC plate being exposed to ammonia fumes before revelation with Dragendorff's reagent.

Fig. 1B reveals that all wash phases (W1, W2, W3 and W4) present a spot at Rf 0.90 that stains positively only after NH_3 treatment, indicating that the alkaloid artifact was washed away during the extraction process, probably because of a higher solubility in diethyl ether than in acidified water.

Prior to derivatization, this spot quenches the plate's UV254 nm fluorescence.

2.2. Identification of the artifact-generating compound

The HPLC analysis of the diethyl ether extract (Supplementary data, Figure 1S) revealed a major peak at R_t = 20.34 min that presents the TLC characteristics of the artifact-generating compound. After scaling-up to preparative HPLC, the compound of interest was isolated, and its purity verified by analytical HPLC (96.5% at UV 254 nm; Supplementary data, Figure 2S).

Direct injection of the isolated compound in ESI-MS (negative ion mode) yielded a major peak at *m/z* 151.01 and its chloride adduct $[M+Cl]^-$ at *m/z* 187.11. This peak could correspond to 2-hydroxy-4-methoxybenzaldehyde (2H4MBZA), to 3-hydroxy-4-methoxybenzaldehyde (isovanillin) or to 4-hydroxy-3-methoxybenzaldehyde (vanillin); these 3 molecules of Mr 152.15 have been previously described in *M. whitei* (Kubo and Kinst-Hori, 1999; Mukonyi and Ndiege, 2001; Watcho et al., 2006). MS/MS fragmentation (Fig. 2) yielded peaks characteristic of these 3 isomers: *m/z* 135.85, $[M-CH_3-H]^-$; *m/z* 122.95, $[M-CHO]^-$ and *m/z* 107.89 for $[M-CH_3-CHO]^-$.

HPLC comparison with authentic standards (R_t isovanillin, 6.75 min; vanillin 7.23 min and 2H4MBZA 20.34 min) led to assign the peak of the artifact-generating compound to 2H4MBZA.

^1H NMR spectral analysis allowed to confirm the structure: the compound of interest yielded chemical shifts (in ppm) at δ = 3.85 (3H, s), δ = 6.42 (1H, d), δ = 6.54 (1H, dd), δ = 7.42 (1H, d), δ = 9.71 (1H, s), δ = 11.48 (1H, s) compatible with 2H4MBZA's structure, as compared to a commercial reference (Sigma-Aldrich, St Louis, USA) and literature data (Mukonyi and Ndiege, 2001).

The content of 2H4MBZA in our sample of *M. whitei* was 0.50% (=5.0 mg/g dry material), as determined by hydrodistillation followed by HPLC. 2H4MBZA has been previously reported in the roots of two Apocynaceae native from India and used in Ayurvedic medicine: *Hemidesmus indicus* (L.) R. Br. ex Schult used to treat skin diseases (2H4MBZA ranging 0.3–3.2 mg/g) and *Decalepis hamiltonii* Wight & Arn. used as a tonifier and "blood purifier" (0.2–4.5 mg/g) (Giridhar et al., 2004; Nagarajan and Rao, 2003; Sircar et al., 2007; Thangadurai et al., 2002). Interestingly, possible presence of alkaloids has been reported in *H. indicus* (Arseculeratne et al., 1985).

The artifact itself was isolated by preparative TLC (Rf = 0.52) and analyzed in ESI-MS positive mode. A major peak was observed at *m/z* 152.11, a mass-to-charge ratio compatible with the protonated $[M+H]^+$ form of 2-hydroxy-4-methoxybenzaldimine (Fig. 3).

The generation of a nitrogen-bearing compound in the course of extraction of *M. whitei*'s roots may explain the conflicting literature data reporting the presence of alkaloids (Ameyaw et al., 2009; Fred-Jaiyesimi and Ogunjobi, 2013; Kerharo, 1974; Pedersen et al., 2008). Unfortunately, these studies do not provide sufficient information regarding the extraction procedure, notably on the eventual use of ammonia.

In preliminary experiments, we tested different alkaloid extraction procedures involving different pH. As none of these extracts (except from those involving ammonia) were positive for Dragendorff reaction, we infer that our sample of *M. whitei* does not