Phytochemistry 153 (2018) 74–78

Contents lists available at ScienceDirect

Phytochemistry

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

Phytochemical profile of the rare, ancient clone *Lomatia tasmanica* and comparison to other endemic Tasmanian species *L. tinctoria* and *L. polymorpha*

Bianca J. Deans[a], Laura Tedone[a,b], Alex C. Bissember[a,**], Jason A. Smith[a,*]

[a] School of Natural Sciences-Chemistry, University of Tasmania, Hobart, Tasmania 7001, Australia
[b] Australian Centre for Research on Separation Science (ACROSS), University of Tasmania, Hobart, Tasmania 7001, Australia

ARTICLE INFO

Keywords:
Lomatia tasmanica
Lomatia polymorpha
Lomatia tinctoria
Proteaceae
Naphthoquinones
Flavonoid glycosides
n-alkanes
n-alcohols
Pressurized hot water extraction

ABSTRACT

An investigation of the previously unexamined ancient Tasmanian clone *Lomatia tasmanica* W. M. Curtis (Proteaceae) and two other endemic species *Lomatia tinctoria* R. Br. and *Lomatia polymorpha* (Labill.) R. Br. was undertaken. This represents the first extensive natural products study in which individual phytochemical components have been isolated and identified from these three *Lomatia* species. Extraction of *L. tasmanica* leaves provided the naphthoquinone juglone (0.34% w/w), and *n*-alkanes nonacosane and heptacosane (0.30% w/w combined). *L. polymorpha* afforded the flavonoid glycosides dihydroquercetin 3-*O*-β-D-xyloside (0.22% w/w) and quercetin 3-*O*-β-D-glucose (0.14% w/w), as well as the naphthalene glucoside 1,4,8-trihydroxynaphthalene-1-*O*-β-D-glucose (0.04% w/w) and 4-*O*-*p*-coumaroyl-D-glucose (0.03% w/w). In addition, both *L. polymorpha* and *L. tinctoria* contained juglone (0.32% w/w and 0.58% w/w, respectively). *L. polymorpha* provided tetracosan-1-ol, hexacosan-1-ol and octacosan-1-ol (0.07% w/w combined), while *L. tinctoria* gave nonacosane (0.13% w/w). Analysis of three individual specimens from each of the three species demonstrated consistency in the respective phytochemical profiles of these populations and tentatively suggests limited intraspecific variation.

1. Introduction

The genus *Lomatia*, belonging to the ancient Gondwanan family Proteaceae, features three endemic Tasmanian trees, namely *L. tasmanica* W.M. Curtis (Proteaceae), *L. tinctoria* R. Br. (Proteaceae) and *L. polymorpha* (Labill.) R. Br (Proteaceae). The endemic Tasmanian tree *L. tasmanica*, also termed King's Holly, is an enigmatic species that is critically endangered and confined to a single population in south-western Tasmania, Australia. Genetic analysis by Lynch and co-workers indicates that *L. tasmanica* is an ancient triploid (3N) clonal species, which exclusively reproduces vegetatively (Brown and Gray, 1985; Lynch et al., 1998), in contrast to *L. tinctoria* and *L. polymorpha*. Fossilized leaves of *L. tasmanica* were dated as a minimum age of 43,600 years old. Accordingly, it is proposed that *L. tasmanica* may represent one of the oldest living plant individuals on Earth (Jordan et al., 1991). Few published studies on *L. tasmanica* have been undertaken. These have been limited to genetic analysis (Lynch et al., 1998) and paleobotany (Jordan et al., 1991) focusing on the evolution and taxonomy of this ancient clone, conservation propagation strategies (Cambecèdes, 1995), ecology (Balmer, 2009; Lynch and Balmer, 2004), and morphology (Cameron, 1981; Curtis, 1967).

Conservation of the critically endangered *L. tasmanica* has been at the forefront of several research investigations in recent years (Cambecèdes, 1995), with propagation strategies a major focus of such efforts, including *via* cuttings and tissue culture (Cambecèdes, 1995). To date, no chemical analysis of *L. tasmanica* has been reported. With this in mind, identifying the phytochemical composition of this rare *Lomatia* species would provide an insight into the chemotaxonomy of this long-living species and this improved understanding may possibly assist in conservation efforts. More specifically, knowledge of *L. tasmanica* phytochemistry may assist these conservation propagation strategies, by shedding light on its physiological functioning and inter-specific ecological interactions.

Few phytochemical investigations on the *Lomatia* genera have been undertaken. Research has been limited to the Australian species *L. myricoides* and *L. fraxinifolia* (Bieleski and Briggs, 2005), and South American species *L. ferruginea* (Mehendale and Thompson, 1975; Bieleski and Briggs, 2005), and *L. hirsuta* (Erazo et al., 1997; Simonsen et al., 2006). In addition, a handful of studies on *L. polymorpha* and *L. tinctoria* have been undertaken. Reports on *L. tinctoria* have included the

* Corresponding author.
** Corresponding author.
E-mail addresses: alex.bissember@utas.edu.au (A.C. Bissember), jason.smith@utas.edu.au (J.A. Smith).

https://doi.org/10.1016/j.phytochem.2018.05.019
Received 3 April 2018; Received in revised form 23 May 2018; Accepted 27 May 2018

isolation of chemical components of seeds and wood (Hooker, 1936; Moir and Thompson, 1973), seed protein composition (Pate et al., 1986), and plant physiology (Leigh et al., 2014). The research on *L. polymorpha* have focused on seed chemical composition (Hooker, 1936) and fungal associations with this species (Lee et al., 2003). Herein, we report the first natural products isolation studies of *L. tasmanica, L. polymorpha* and *L. tinctoria* supported by extensive spectroscopic evidence. This was achieved with the aid of two complementary techniques: traditional maceration extraction; and a newly developed pressurized hot water extraction (PHWE) method for natural products isolation (Deans et al., 2016, 2017, 2018a, 2018b; Just et al., 2015a, 2015b, 2016).

2. Results

Because the leaves and stems of *L. tasmanica, L. tinctoria* and *L. polymorpha* typically blacken or discolor when cut or damaged, fresh (rather than dried) leaves were used in this study to reduce the likelihood of decomposition. The leaves of all *Lomatia* species were initially extracted via maceration using diethyl ether then subjected to PHWE. Maceration with diethyl ether was first undertaken as it was anticipated this solvent would disrupt the waxy coating of the leaves which can affect the efficiency of extractions with water and other polar protic solvents (Deans et al., 2018a; Olivier et al., 2016).

2.1. Lomatia tasmanica

Maceration of *L. tasmanica* provided the naphthoquinone juglone (**1**) (0.34% w/w) (Fig. 1). The ^{1}H and ^{13}C NMR spectroscopic and MS data (see: Supporting Information) were consistent with equivalent data previously reported in the literature (de Freitas Araujo et al., 2009). In addition, this extract also contained a chromatographically inseparable mixture of *n*-alkanes (0.30% w/w). ^{1}H NMR and GC-MS analysis of this mixture was consistent with nonacosane ($C_{29}H_{60}$) as the major component and heptacosane ($C_{27}H_{56}$) as the minor component (see: Supporting Information) (Lytovchenko et al., 2009). Neither of these long-chain alkanes has previously been reported from any *Lomatia* species. This suggests that these hydrocarbons represent major components of the epicuticular wax coating of the characteristically robust Proteaceae leaves. The PHWE extract provided glucose as a mixture of α-and β-anomers (see: Supporting Information).

Juglone (**1**), as well as other naphthoquinone pigments, have previously been isolated from numerous *Lomatia* species, including the Tasmanian endemic *L. tinctoria* (Moir and Thompson, 1973). A range of related naphthoquinones have been isolated from other *Lomatia* species, including: lomatiol (**2**) (Fig. 1), which has been identified in the seeds of both endemic Tasmanian *L. tinctoria* and *L. polymorpha* (Hooker, 1936), and naphtharazin (**3**) from various species (Moir and Thompson, 1973). Perhaps the presence of a single naphthoquinone in *L. tasmanica* reflects the primitive and ancient position of *L. tasmanica* within the lineage, as an evolved trend of increased pigment composition complexity is observed within later descendants within the genus (Moir and Thompson, 1973). While it is possible that juglone **1** can be produced during extraction from its glycoside 1,4,8-trihydroxynaphthalene-1-O-β-D-glucose **6** (Duroux et al., 1998), extraction of fresh leaves from a representative specimen of *L. tasmanica* with diethyl ether for 5 min in an oxygen-free atmosphere did not detect the presence of glycoside **6**.

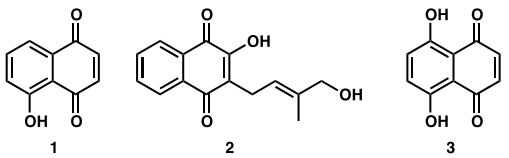

Fig. 1. Juglone (**1**), lomatiol (**2**), and naphtharazin (**3**).

This indicates that naphthoquinone **1** is not an artefact of extraction/isolation and is a true natural product.

Juglone (**1**), like many naphthoquinones, exhibits antifungal (Meazza et al., 2003; Wianowska et al., 2016), antimycobacterial activity (Tran et al., 2004), and is a reported antifeedant towards insects (Akhtar et al., 2012). In addition, juglone is reported to affect plant development, including the promotion of cell division, cell elongation and root formation (Compton and Preece, 1988). Naphthoquinone **1** also has reported allelopathic activity and toxicity towards plants, with its bioactivity as a phytotoxin first reported in 1928 (Davis, 1928). It has been reported that plants may release juglone to stunt the growth of competing plants found in close proximity; and may serve to provide a chemical ecological advantage (eg. Topal et al., 2006). The reported bioactivity of juglone may provide some insight into the chemical ecological defenses that *L. tasmanica* may have developed (perhaps against fungus), as well as interactions and environmental pressures that *L. tasmanica* may have been exposed to over time.

2.2. Lomatia polymorpha

Maceration of *L. polymorpha* provided juglone (**1**) (0.32% w/w), and a mixture of three aliphatic *n*-alcohols (0.07% w/w), which were identified as tetracosanol ($C_{24}H_{50}O$), hexacosanol ($C_{26}H_{54}O$) and octacosanol ($C_{28}H_{58}O$) in addition to heptacosane and nonacosane. The respective GC-MS and retention data were consistent with the equivalent data in National Institute of Standards and Technology (NIST) reference libraries (see: Supporting Information). GC-MS analysis of the purified fraction also indicated the presence of 1-pentacosanol, 1-heptacosanol, and 1-nonacosanol in small quantities (see: Supporting Information). Notably, this represents the first isolation of *n*-alcohols from *Lomatia* leaves.

The PHWE extract provided dihydroquercetin 3-*O*-β-D-xyloside (**4**) (0.22% w/w), quercetin 3-*O*-β-D-glucose (**5**) (0.14% w/w), 1,4,8-trihydroxynaphthalene 1-*O*-β-D-glucose (**6**) (0.04% w/w), and 4-*O*-*p*-coumaroyl-D-glucose (**7**) as a mixture of diastereoisomers, (0.03% w/w) (Fig. 2). The respective ^{1}H and ^{13}C NMR spectroscopic and MS data for compounds **4–6** were consistent with equivalent data previously reported in the literature (see: Supporting Information) (Baderschneider and Winterhalter, 2001; Fernandez et al., 2005; Son, 1995). Flavonoids have previously been isolated from *L. dentata* (Moir and Thompson, 1973). The NMR spectroscopic data and MS data for ester **7** was consistent with the proposed structure (see: Supporting Information). However, only limited NMR spectroscopic data has been reported for this known compound (Birkofer et al., 1969; Kuraishi et al., 1984).

2.3. Lomatia tinctoria

Maceration of *L. tinctoria* provided juglone (**1**) (0.58% w/w) and nonacosane ($C_{29}H_{60}$) (0.13% w/w), as supported by ^{1}H NMR and GC-MS data (see: Supporting Information) (Lytovchenko et al., 2009). GC-MS analysis of the crude diethyl ether extract also indicated the

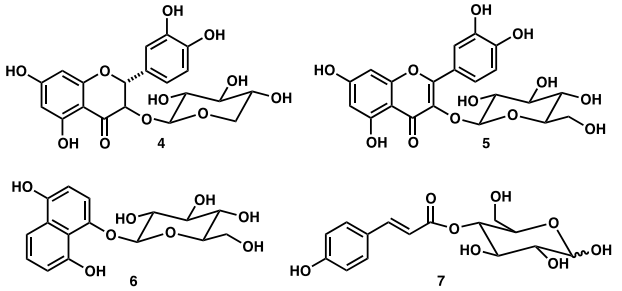

Fig. 2. Dihydroquercetin 3-*O*-β-D-xyloside (**4**), quercetin 3-*O*-β-D-glucose (**5**), 1,4,8-trihydroxynaphthalene-1-*O*-β-D- glucose (**6**), and 4-*O*-*p*-coumaroyl-D-glucose (**7**).