Stereochemistry of Lignans in *Phaleria macrocarpa* (Scheff.) Boerl.

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Z. Naturforsch. **63c**, 13–16 (2008); received July 20/October 25, 2007

Phaleria macrocarpa (Scheff.) Boerl., a member of the Thymelaeaceae, is traditionally used in Indonesia as medicinal plant against cancer. In this context, we isolated the lignans pinoresinol, lariciresinol and matairesinol from different parts of this plant. The enantiomeric composition of these lignans was determined by chiral column analysis. Pinoresinol and lariciresinol were mixtures of both enantiomers with (79 ± 4)% and (55 ± 6)% enantiomeric excess for the (–)-enantiomers, respectively, whereas matairesinol was found as pure (+)-enantiomer.

Key words: Phaleria macrocarpa, Lignan, Enantiomeric Composition

Introduction

Phaleria macrocarpa (Scheff.) Boerl., a member of the Thymelaeaceae (Hou, 1960), is used traditionally in Indonesia to treat diabetes, liver diseases, vascular problems, and cancers (Harmanto, 2001). The parts of this plant used for medicinal purposes are the stem, leaves and fruits. Sumastuti and Sonlimar (2002) reported that the leaf and fruit extract of *P. macrocarpa* inhibits the proliferation of human uterine cervical carcinoma cells. Furthermore, Lisdawati (2002) reported that *P. macrocarpa* contains alkaloids, terpenoids, flavonoids, and lignans.

Lignans are a class of secondary metabolites which are biosynthesized by dimerization of phenylpropanoid units. Two molecules of coniferyl alcohol are coupled to pinoresinol. A so-called dirigent protein leads to the exclusive formation of (+)-pinoresinol in *Forsythia intermedia* (Davin *et al.*, 1990). Dirigent proteins or genes encoding them were also detected in other plant species leading to the assumption that the enantiomeric purity of the lignans is already determined by the coupling of coniferyl alcohol molecules (Xia *et al.*, 2000; Davin and Lewis, 2003). (+)-Pinoresinol is reduced via (+)-lariciresinol to (–)-secoisolariciresinol by pinoresinol-lariciresinol reductase and subsequently oxidized to (–)-matairesinol (Umezawa *et al.*, 1991; Dinkova-Kostova *et al.*, 1996; Xia *et al.*, 2001; Okunishi *et al.*, 2004; von Heimendahl *et al.*, 2005; Youn *et al.*, 2005; Moinuddin *et al.*, 2006). In contrast to the formation of (–)-matairesinol in *Forsythia* species the opposite lignan enantiomers can be found in other species, especially of the Thymelaeaceae familiy (Umezawa *et al.*, 1997; Umezawa, 2003). Furthermore studies on the enantiomeric composition of lignans in different plant species showed that in many cases matairesinol is enantiomeric pure while pinoresinol, lariciresinol and secoisolariciresinol are mixtures of both enantiomers in various compositions.

In this paper, we report on the occurrence of lignans and their enantiomeric composition from different parts of *P. macrocarpa*.

Materials and Methods

Materials

Plant material of *P. macrocarpa* was collected from Lombok Island, Indonesia, in January and August 2006. A voucher specimen was deposited at the Institut für Entwicklungs- und Molekularbiologie der Pflanzen, Heinrich-Heine-Universität Düsseldorf, Germany. All solvents and chemicals were of reagent or HPLC grade. Racemic pinoresinol was synthesized according to Xia *et al.* (2001). Lariciresinol and matairesinol were from Arbonova (Turku, Finland).

Instrumentation

HPLC analyses were carried out by using an HPLC-PDA system from Thermoquest (Egels-

0939–5075/2008/0100–0013 $ 06.00

bach, Germany) which consists of a degasser, autosampler AS1000, photodiode array detector Spectra Systems UV6000LP and a pump P2000. HPLC columns were Hypersil HyPurity™ Elite C18 (Thermoquest), GromSil 120 ODS-5 ST (Grom Company, Rottenburg-Hailfingen, Germany), Chiracel OC and Chiracel OD-H (Daicel, Eschborn, Germany). HPLC-MS was performed with a mass spectrometer from Finnigan LCQ Deca XP (Thermo Finnigan, Dreieich, Germany) coupled to an Agilent HP1100 HPLC system (Agilent, Waldbronn Germany).

Lignan extraction

Lignan extraction was modified according to Wichers *et al.* (1990). 0.20 g powdered, freeze-dried cells were suspended in 2 ml methanol and incubated in an ultrasonic bath on ice two times for 30 s each with a 30 s break. The pH value was adjusted to 5.0 by the addition of diluted phosphoric acid after addition of 8 ml bidistilled water. 1 mg β-glucosidase (from almonds, $\geq$1000 U/mg, Roth, Karlsruhe, Germany) was added. After incubation at 35 °C for 1 h the samples were extracted 3 times with 8 ml ethyl acetate each. The combined ethyl acetate phases were dried under vacuum and the residue was resolved in an appropriate volume of methanol for HPLC analysis.

HPLC analysis

HPLC analysis was conducted by using a Hypersil HyPurity™ Elite C18 column (Thermoquest) at a flow rate of 1.1 ml/min with an elution system employing water containing 0.01% (v/v) phosphoric acid (A) and acetonitrile (B). The following gradient was used: 20.5% B for the first 35 min, 20.5–65% B in 10 min, 65% B for 2 min, 65–20.5% B in 4 min, 20.5% B for 4 min. Lignans were identified by comparison of retention times and UV spectra with authentic standards. Lignans were collected by reversed phase HPLC with a semi-preparative Grom-Sil column and subjected to mass spectrometry analysis. HPLC-MS was conducted by using an Eurospher 100-C18 (5 μm; 227 mm $\times$ 2 mm) column (Knauer, Berlin, Germany) with acetonitrile and methanol as solvents. The enantiomeric composition of lignans was analyzed by chiral column HPLC according to Umezawa *et al.* (1990, 1994). Ethanol (A) and *n*-hexane (B) were used as solvents at a flow rate of 0.5 ml/min. The separation of the enantiomers of pinoresinol and lariciresinol was achieved according to von Heimendahl *et al.* (2005). The separation of the enantiomers of matairesinol was conducted by using a Chiracel OD-H column with 85% *n*-hexane (containing 1% acetic acid) and 15% ethanol as solvents. The flow rate was 0.5 ml/min. The detection wavelength was 280 nm. Identification of the substances was achieved by comparison of retention times and UV spectra with authentic standards.

Results and Discussion

Pinoresinol, lariciresinol and/or matairesinol could be detected in the different organs of *Phaleria macrocarpa* by HPLC in comparison to authentic standards (Table I). The fragmentation pattern of the lignans isolated from *P. macrocarpa* was identical to the one of authentic standards in HPLC-MS. Especially wood and root were rich in pinoresinol, but contained also lariciresinol and matairesinol. The seeds contained a smaller amount of pinoresinol as main lignan and lariciresinol; matairesinol could not be detected in the seeds. Larciresinol was the only lignan which could be detected in the bark, leaf and fruit flesh. Since the wood of *Phaleria macrocarpa* was the richest source of lignans with respect to the type of lignan as well as the amount we investigated the enantio-

Table I. Lignans in *Phaleria macrocarpa* (Scheff.) Boerl.

Plant material	Lignan content [mg/g dry weight]		
	Pinoresinol	Lariciresinol	Matairesinol
Root	3.40	1.53	1.56
Bark	–	0.88	–
Wood	2.24	1.77	0.80
Leaf	–	0.50	–
Fruit flesh	–	0.64	–
Seed	0.80	0.26	–

(–) not detected.

Table II. Enantiomeric composition of lignans isolated from extracts of the wood of *P. macrocarpa* (Scheff.) Boerl. (mean ± standard deviation from 4 chiral column analyses).

Lignan	(+)-Enantiomer (%)	(–)-Enantiomer (%)
Pinoresinol	10.5 ± 1.7	89.5 ± 1.7
Lariciresinol	22.5 ± 3.1	77.5 ± 3.1
Matairesinol	100	0

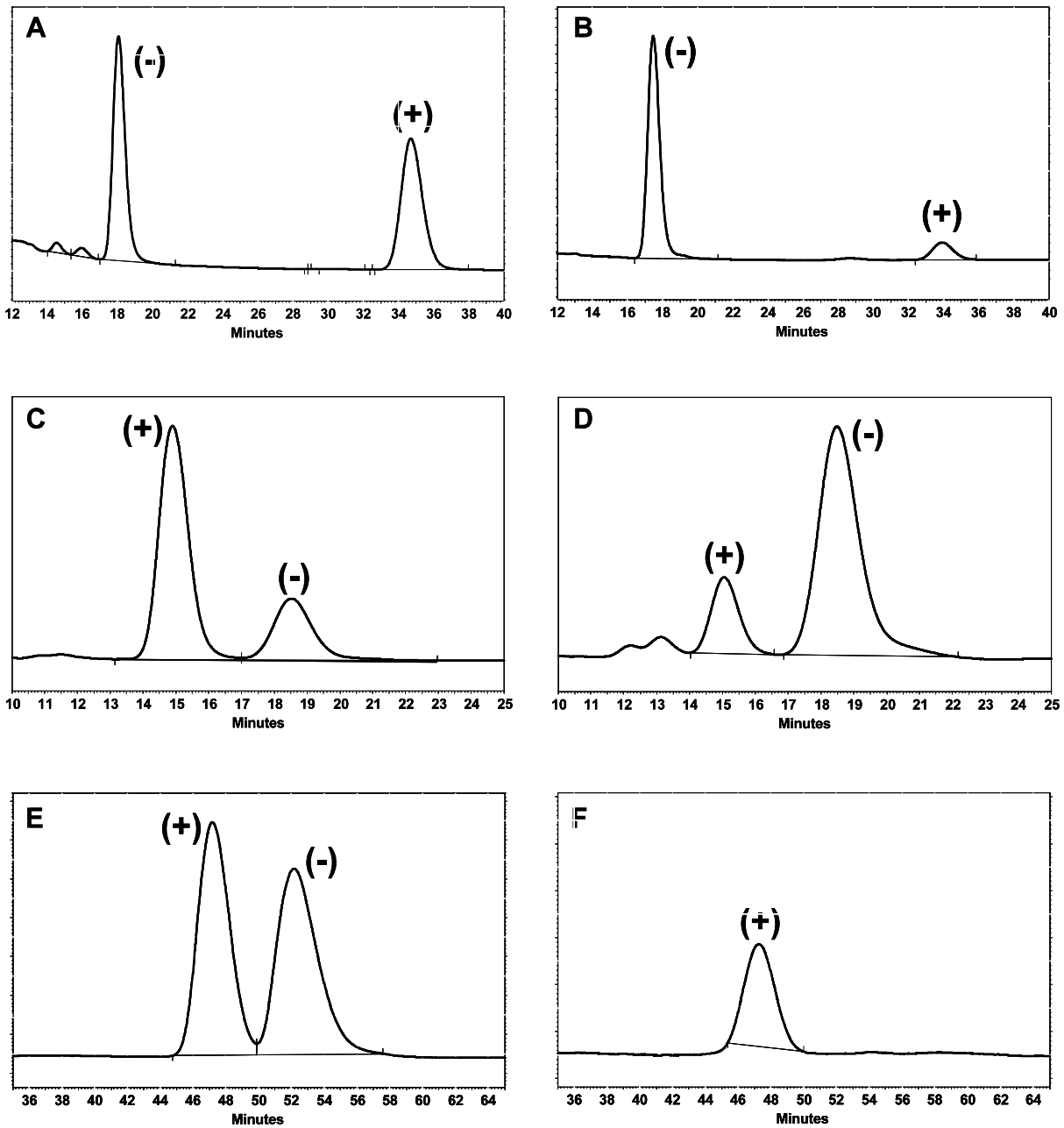

Fig. 1. Chiral column HPLC analysis of (A) racemic pinoresinol; (B) pinoresinol isolated from extracts of *P. macrocarpa*; (C) racemic lariciresinol; (D) lariciresinol isolated from extracts of *P. macrocarpa*; (E) racemic matairesinol; (F) matairesinol isolated from extracts of *P. macrocarpa*.

meric composition of pinoresinol, lariciresinol and matairesinol which were collected from reversed phase HPLC and submitted to chiral column HPLC after purification (Fig. 1, Table II). Whereas pinoresinol and lariciresinol were mixtures of both enantiomers, matairesinol accumulated as pure (+)-enantiomer. These data confirm the results from Umezawa *et al.* (1997) and Umezawa (2003) who described that in Thymelaeaceae species, to which *Phaleria macrocarpa* belongs, matairesinol

occurs as pure (+)-enantiomer while pinoresinol, lariciresinol and secoisolariciresinol are mixtures of both enantiomers in various compositions.

Conclusion

Biosynthetically simple lignans such as pinoresinol, lariciresinol, and matairesinol were isolated from different organs of *Phaleria macrocarpa* (Scheff.) Boerl. The occurrence of these lignans was confirmed by comparison of the retention times, UV spectra and mass spectra with authentic standards. Pinoresinol and lariciresinol were mixtures of both enantiomers with (79 ± 4)% enantiomeric excess and (55 ± 6)% enantiomeric excess for the (−)-enantiomers, respectively, whereas matairesinol was found as optically pure (+)-enantiomer. Isolation and characterization of a cDNA which probably encodes a pinoresinol-lariciresinol reductase (PLR) from *P. macrocarpa* is in progress.

Acknowledgement

Financial support from DAAD for A. Saufi (no. A/03/41187) is gratefully acknowledged. We thank Ru Angelie Edrada for performing the LC-MS measurements.

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