Phytochemistry 70 (2009) 262–269

Contents lists available at ScienceDirect

Phytochemistry

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

The chain length of lignan macromolecule from flaxseed hulls is determined by the incorporation of coumaric acid glucosides and ferulic acid glucosides

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ARTICLE INFO

Article history:
Received 23 September 2008
Received in revised form 6 December 2008
Available online 18 January 2009

Keywords:
Flaxseed
Lignan
Lignan macromolecule
Secoisolariciresinol
p-Coumaric acid glucoside
Ferulic acid glucoside
Extinction coefficient
Chain length

ABSTRACT

Lignan macromolecule from flaxseed hulls is composed of secoisolariciresinol diglucoside (SDG) and herbacetin diglucoside (HDG) moieties ester-linked by 3-hydroxy-3-methylglutaric acid (HMGA), and of *p*-coumaric acid glucoside (CouAG) and ferulic acid glucoside (FeAG) moieties ester-linked directly to SDG. The linker molecule HMGA was found to account for 11% (w/w) of the lignan macromolecule. Based on the extinction coefficients and RP-HPLC data, it was determined that SDG contributes for 62.0% (w/w) to the lignan macromolecule, while CouAG, FeAG, and HDG contribute for 12.2, 9.0, and 5.7% (w/w), respectively.

Analysis of fractions of lignan macromolecule showed that the higher the molecular mass, the higher the proportion of SDG was. An inverse relation between the molecular mass and the proportion (%) CouAG + FeAG was found. Together with the structural information of oligomers of lignan macromolecule obtained after partial saponification, it is hypothesized that the amount of CouAG + FeAG present during biosynthesis determines the chain length of lignan macromolecule.

Furthermore, the chain length was estimated from a model describing lignan macromolecule based on structural and compositional data. The average chain length of the lignan macromolceule was calculated to be three SDG moieties with CouAG or FeAG at each of the terminal positions, with a variation between one and seven SDG moieties.

1. Introduction

Lignans are phenolic compounds, which, by definition, are composed of two propyl-benzene moieties, which are linked by a bond between the 8 and the 8′ position (Moss, 2000). The propyl-benzene structures, coniferyl alcohols, are biosynthesized through the phenylpropanoid pathway and stereospecifically dimerized into the lignan pinoresinol (PINO) (Davin et al., 1997).

Subsequently, reduction, oxidation, dehydrogenation and addition reactions lead to the formation of a broad range of lignans, which are present in both aglyconic and in glycosylated forms (Ford et al., 2001; Hano et al., 2006; von Heimendahl et al., 2005; Youn et al., 2005). Lignans are of interest because of their potential (anti)-estrogenic and antioxidant properties (Eklund et al., 2005; Wang, 2002).

Secoisolariciresinol diglucoside (SDG) is the most abundant lignan present in flaxseed (Johnsson et al., 2000). It is synthesized from PINO by the action of pinoresinol–lariciresinol reductase (PLR) (von Heimendahl et al., 2005), followed by glucosylation by an UDPG:glucosyltransferase (Ford et al., 2001). The formation of SDG takes place in the outer layer of the seed (Hano et al., 2006). Therefore, the concentration of SDG found in flaxseed hulls is higher than that of whole seeds (Madhusudhan et al., 2000). The lignans in flaxseeds (hulls) are part of an oligomeric structure, called lignan macromolecule. Within this lignan macromolecule, SDG is ester-linked by 3-hydroxy-3-methylglutaric acid (HMGA) (Kamal-Eldin et al., 2001; Klosterman and Smith, 1954). Also herbacetin diglucoside (HDG), ferulic acid glucoside (FeAG) and *p*-coumaric acid glucoside (CouAG) are part of the lignan macromolecule (Johnsson et al., 2002; Struijs et al., 2007). The chemical structures of the constituents of lignan macromolecule are shown in Fig. 1.

Analysis of seeds at different developmental stages showed that hardly any free SDG is present in developing seeds. Almost directly after its formation, SDG is incorporated within the lignan macromolecule (Ford et al., 2001; Hano et al., 2006). Oligomers of SDG and HMGA are formed by ester-linkage with CoA-activated HMGA (Ford et al., 2001). Molecules consisting of one or two SDG moieties with one, two or three HMGA moieties have been identified (Ford et al., 2001). Just like SDG, CouAG and FeAG are incorporated in an alkali labile structure in early stages of seed development (Ford et al., 2001; Hano et al., 2006).

Only limited data about the composition and size of lignan macromolecule are available. In 2001, a model of lignan

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doi:10.1016/j.phytochem.2008.12.015

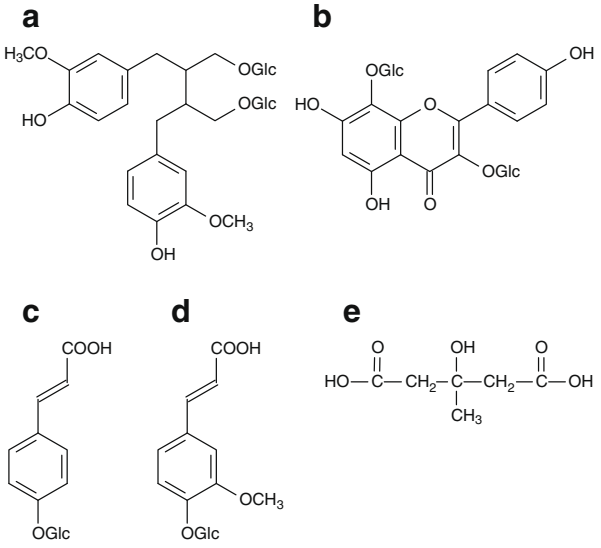

Fig. 1. The chemical structures of the constituents of lignan macromolecule from flaxseed hulls. a = SDG, b = HDG, c = CouAG, d = FeAG and e = HMGA.

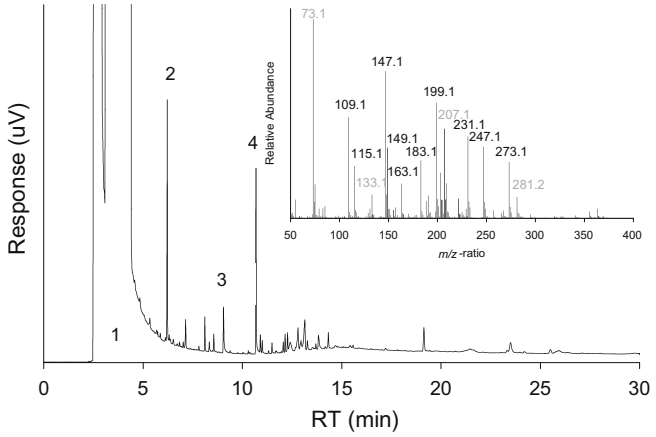

Fig. 2. GC profile of fully saponified lignan macromolecule after derivatisation. 1 = pyridine, 2 = derivatizing agent, 3 = glutaric acid (internal standard), 4 = HMGA. In the insert the GC-MS spectrum of peak 4. *m*/*z*-ratios ($[M + H]^+$) marked gray are also present in other fractions eluting at different retention times and are, therefore, not originating from HMGA.

macromolecule consisting of five SDG units being esterified with four HMGA residues was published (Kamal-Eldin et al., 2001). However, in later studies, variation in the proportions of constituents was observed between fractions of lignan macromolecule separated by reversed-phase solid-phase extraction (Johnsson et al., 2002; Strandas et al., 2008), indicating that lignan macromolecule represents an array of molecules with varying composition. In addition, it has been shown that the flavonoid HDG links, just like SDG, within lignan macromolecule via HMGA (Struijs et al., 2007), while CouAG and FeAG are linked directly to the glucosyl moieties of SDG through their carboxylic groups (Struijs et al., 2008).

More precise data about the composition and size of lignan macromolecule could give direction to further research on the biosynthesis of lignan macromolecule. The aim of present research is to investigate the composition and size of lignan macromolecule from flaxseed hulls in order to be able to determine the correlation between the composition and the size of the molecules.

2. Results and discussion

2.1. Quantification of the constituents of lignan macromolecule

An extract of lignan macromolecule from flaxseed hulls was obtained by extraction with 63% aq. EtOH (Struijs et al., 2007). Monomeric units of lignan macromolecule were obtained by treating the lignan macromolecular extract with 75 mM NaOH (Struijs et al., 2007). The components described to be part of lignan macromolecule from flaxseed hulls, can be divided into two groups: the phenolic constituents (see later) and the linker molecule HMGA. Fig. 2 shows the GC profile of HMGA from fully saponified lignan macromolecule. To confirm that peak 4 corresponded to HMGA, it was analyzed by GC–MS. In the insert of Fig. 2, the MS spectrum of peak 4 (RT = 10.9 min) is shown. By comparing the spectrum with the spectrum of an authentic standard of HMGA and with literature values (Ford et al., 2001), this peak was identified as HMGA. The concentration HMGA was calculated as 111 ± 13 μg/mg macromolecule. This number corresponds with the amount determined on the basis of the recovered weights of HMGA isolated from lignan macromolecule as reported previously (Prasad, 2004).

Besides the 11.1% (w/w) of HMGA, lignan macromolecule consisted of phenolic compounds, mainly SDG, CouAG, FeAG and HDG. To be able to determine the proportion of each of these phenolic constituents, they were purified by preparative RP-HPLC (Struijs et al., 2007), and their molar extinction coefficients were determined, as these data have not been reported in literature.

The molar extinction coefficients at 280 nm of purified SDG, CouAG, FeAG and HDG are given in Table 1. In this table, also the purity of the compounds, based on NMR signals is given (see Struijs et al., 2007 for NMR). The purity of the compounds was found to be close to 90% or higher.

On a molar basis, SDG was found to be the most abundant constituent, followed by CouAG, FeAG, and HDG as shown in Table 2. Recalculation of the molar proportions of the phenolic compounds into weight ratios showed that lignan macromolecule consists of 62.0% (w/w) SDG, 5.7% HDG, 12.2% CouAG and 9.0% FeAG. These

Table 1
Molar extinction coefficients of the four main phenolic constituents of lignan macromolecule.

Constituent	Purity	Molar extinction coefficient at 280 nm (M^{-1} cm^{-1})	SD absolute (%)	λ_{max} (nm)
CouAG	92%	19474	1043 (5.4%)	297
FeAG	98%	12133	292 (2.4%)	291 + 316
HDG	89%	10347	158 (1.5%)	271
SDG	100%	5838	201 (3.4%)	281

Table 2
Molar proportions and weight percentages of the constituents of lignan macromolecule.

Constituent	Molar proportions[a] of phenolic constituent (%)	±SD	Weight percentage[b] (%, w/w)	±SD
CouAG	23.1	±2.8	12.2	±1.5
FeAG	15.6	±0.9	9.0	±0.5
HDG	5.6	±0.4	5.7	±0.4
SDG	55.7	±2.0	62.0	±2.2
HMGA			11.1	±1.3

[a] Determined based on peak area of the RP-HPLC profiles (not shown; see Struijs et al. (2007) and the molar extinction coefficient (Table 1).
[b] The weight percentage (%, w/w) of HMGA was determined by GC. The weight percentage (%, w/w) of phenolic constituents were calculated from molar proportions.