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Note

Characterization of *Jatropha curcas* lignins

Masaomi Yamamura[1], Kinya Akashi[2], Akiho Yokota[2], Takefumi Hattori[1], Shiro Suzuki[1], Daisuke Shibata[3], Toshiaki Umezawa[1,4,*]

[1] Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan; [2] Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan; [3] Kazusa DNA Research Institute, Kisarazu, Chiba 292-0818, Japan; [4] Institute of Sustainability Science, Kyoto University, Uji, Kyoto 611-0011, Japan
* E-mail: tumezawa@rish.kyoto-u.ac.jp Tel: +81-774-38-3625 Fax: +81-774-38-3682

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Abstract Lignins of *Jatropha curcas* organs were qualitatively and quantitatively characterized by thioglycolic acid, thioacidolysis, and nitrobenzene oxidation methods. The lignin content of the seed coat was 49.4%, and was the highest among various organs of the plant, while the stem had 15.9% lignin, within the range of usual lignin contents of angiosperm trees. Lignin aromatic components of all organs were composed of guaiacyl and syringyl units. Nitrobenzene oxidation indicated that the ratios of syringyl to guaiacyl lignins in the fruit coat and stem were higher than those in other organs. This study provides basic data for the total utilization of *J. curcas* wood.

Key words: *Jatropha curcas*, lignin, nitrobenzene oxidation, thioacidolysis, thioglycolic acid method.

Biodiesel is becoming increasingly economically important due to diminishing petroleum reserves and the environmental consequences of exhaust gases from petroleum-fuelled engines (Berchmans and Hirata 2008). Palm oil, which is produced by oil palms grown in tropical countries, can be used to produce biodiesel. However, palm oil has long been widely used as a cooking oil. The world's palm oil production is 38.5 million tons a year in 2007 (Lam et al. 2009), and increased consumption of palm oil for biodiesel could cause supply instability and price fluctuations of vegetative oils (Basiron 2007; Carter et al. 2007; Lam et al. 2009). In addition, lands suitable for oil palm plantations overlap tropical forests, thereby expansion of the plantations may induce the conflict with the preservation of natural forests in tropical countries such as Malaysia and Indonesia.

In this context, the development of an alternative vegetative oil source is being necessitated. Fruits of *Jatropha curcas* L. (Euphorbiaceae), which is also known as physic nut or purging nut, contain a number of seeds that include large amounts of oil. The seed yield is about 7 ton ha^{-1} $year^{-1}$ (Sudhakar et al. 2012), and the production of *J. curcas* oil is about 2.2–2.7 ton ha^{-1} $year^{-1}$ (Sudhakar et al. 2012), which is comparable to that of palm oil production (3.7 ton ha^{-1} $year^{-1}$; Sumathi et al. 2008). The seed and/or oil were found to be toxic to animals. Hence, their use as a human food or animal feed source are presently limited (Makkar et al. 1998), avoiding conflict with food production and perturbation of food oil prices (Gomaa et al. 2011). In addition, *J. curcas* is easy to establish, and grows relatively quickly (Makkar et al. 1998). The plant has an excellent adaptation capacity to a large variety of soil conditions (Makkar and Becker 2009), and a geographic distribution of *J. curcas* is broader than that of oil palm (King et al. 2009). For all of these reasons, *J. curcas*, which is a native of tropical America, now thrives in many tropical and sub-tropical regions in Asia and Africa (Openshaw 2000).

J. curcas trees are trimmed to ca. 2 m in height to facilitate the harvesting of the fruits (Iiyama 2007). As a result, *J. curcas* oil production is accompanied by the concomitant production of 10 ton ha^{-1} $year^{-1}$ of branches (Iiyama 2007). The yield of the woody branches is almost similar to that of fast growing trees in temperate zone, e.g., 5–12 ton ha^{-1} $year^{-1}$ for Japanese cedar (*Cryptomeria japonica*) (Shutou and Nakane 2004) and poplar (*Populus trichocarpa*) (Cannell 1989). Thus, the utilization of the trimmed braches is critically important for increasing the economical efficiency of *J. curcas* plantations. To exploit the branches as lignocellulosic raw materials for the production of pulp, paper, wooden boards, and biofuels, it is necessary to characterize lignins in the branches, because both lignin contents and structures, which vary among plant species and among organs within a single plant species (Whetten et al. 1998), affect largely the utilization of lignocellulosic materials. For example, lignin is to be removed in pulping processes. In

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addition, lignin impedes access of hydrolyzing enzymes to the polysaccharides, thereby inhibiting enzymatic saccharification of plant biomass (Wang et al. 2011). In these processes less lignin can be beneficial (Chen and Dixon 2007). In relation to lignin structures, plants with higher syringyl lignin contents are more easily delignified in kraft pulping (Chiang and Funaoka 1990). On the other hand, lignin is an important biofuel, because it has higher carbon contents and heating values than polysaccharides. In fact, lignin degradation products in the black-liquors, which are effluents from kraft-pulp mills, are being exploited as a by-product fuel in the mills. The amounts were equivalent to 5.4 million kl of crude oil in Japan in 2008 (http://www.hokuetsu-kishu.jp/environment/pdf/2008_ja_all.pdf).

Despite their potential importance, little has been known about the lignins of *J. curcas*. The lignin contents of seed (Makkar et al. 1998; Makkar and Becker 2009; Manurung et al. 2009), fruit coat (Jingura et al. 2010), leaf (Ruíz-Valdiviezo et al. 2010), and stem (Vaithanomsat and Apiwatanapiwat 2009) of *J. curcas* have been reported. However, there were no reports of detailed characterizations of the lignins in *J. curcas* using chemical degradation methods, such as thioacidolysis and nitrobenzene oxidation or by nuclear magnetic resonance spectrometry. Herein, we characterized the lignins of various organs of *J. curcas*.

J. curcas L. cv. IP-2P were grown in a growth chamber under a light intensity of 200 μmol photon $m^{-2} s^{-1}$ and 12-h light/12-h dark illumination regime, with a temperature and humidity cycle of 28/21°C and 65/50%, respectively. Six-month-old plants were divided into the following parts: hermaphrodite flower, male flower, stem, leaf, fruit coat, seed, seed coat, and kernel (Figure 1). Each sample was individually freeze-dried and powdered with a TissueLyser (Qiagen GmbH, Hilden, Germany) for 3 min at 25 Hz at room temperature. The powders thus obtained were extracted twenty times with methanol for 5 min at 60°C. Then, the powders were successively extracted with hexane and distilled water, and freeze-dried. The dried powders were subjected to lignin analyses: thioacidolysis, nitrobenzene oxidation, and thioglycolic acid methods. The hermaphrodite and the male flower samples were not submitted to thioacidolysis because of their limited amounts. Secondary xylem of a one-year-old stem of the plant was also analyzed by the thioglycolic acid method.

First, each sample was subjected to thioacidolysis (Nakatsubo et al. 2008; Yamamura et al. 2011) to confirm the presence of lignin by identifying phenyltrithioethylpropane compounds (Figure 2), which are derived specifically from *β-O*-4 substructures of lignin polymers. The detection of the diagnostic monomers clearly indicated the presence of lignin in all analyzed samples (Figure 3). The yields of the monomers

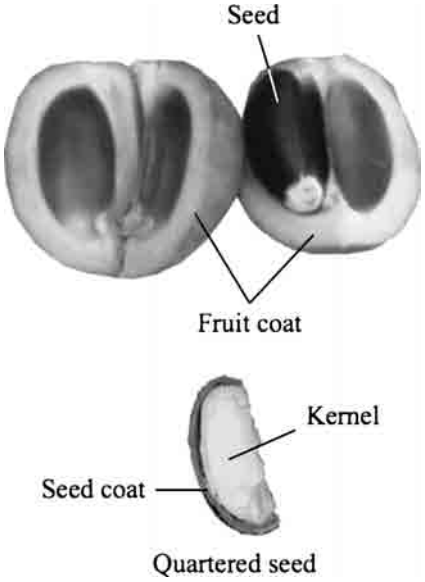
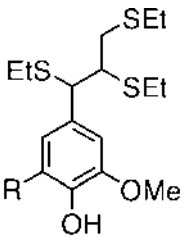


Figure 1. Section of fruit and seed of *Jatropha curcas*.



R=H, Guaiacyltrithioethylpropane

R=OMe, Syringyltrithioethylpropane

Figure 2. Thioacidolysis products.

from stem, fruit coat, seed, seed coat, and seed cake were higher than those from leaf, kernel, and squeezed kernel. Especially, the yields from kernel and squeezed kernel were small and detected only by selected ion monitoring (SIM) mass spectrometry (Figure 3). In addition, the aromatic substitution patterns of the *β-O*-4 substructure-derived monomers revealed that the lignins were composed of guaiacyl and syringyl units. Recently, catechyl lignin that is formed by polymerization of an unusual monomer, caffeyl alcohol, was detected in some plant seeds (Chen et al. 2012). However, phenyltrithioethylpropane monomers from catechyl lignin units were not detected in *J. curcas* seeds and seed coats under the used analytical condition (data not shown).

To determine the lignin aromatic components not only of *β-O*-4 substructure which is obtained by the thioacidolysis but also of other substructures, each organ of *J. curcas* was analyzed by the microscale nitrobenzene oxidation method (Yamamura et al. 2010, 2011). GC-MS analysis of the nitrobenzene oxidation products

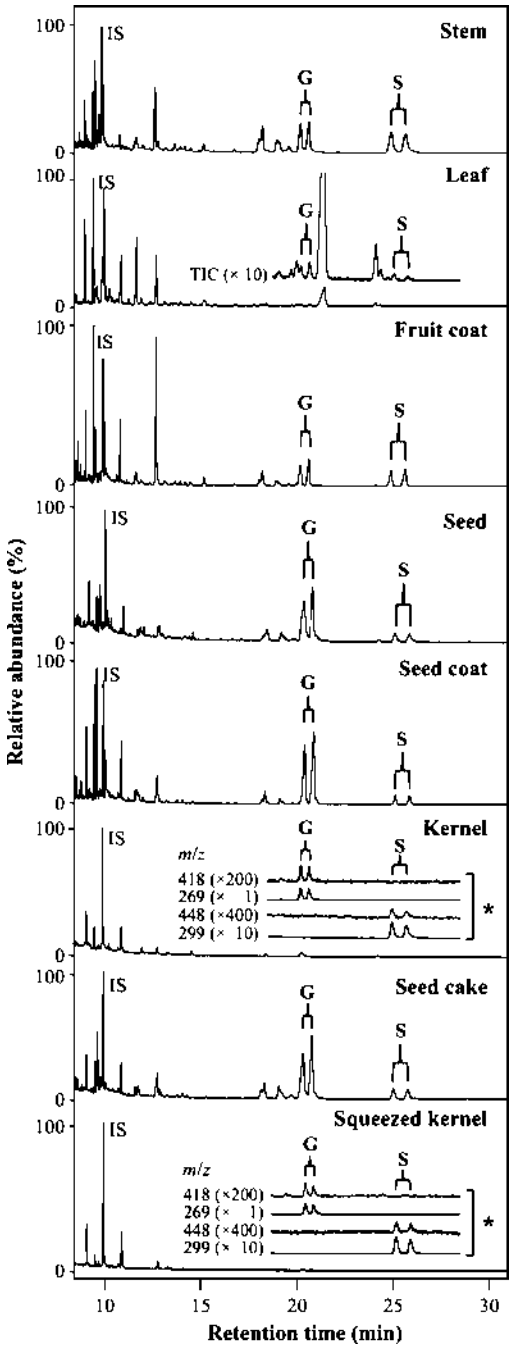


Figure 3. Total ion chromatograms of thioacidolysis products (trimethylsilyl ethers). G: guaiacyltrithioethylpropane, S: syringyltrithioethylpropane. Two peaks of G and S are *erythro* and *threo* isomers. TIC: Total ion chromatogram. * SIM chromatogram (G: *m/z* 418, $[M]^+$ and base ion, 269; S: *m/z* 448, $[M]^+$ and base ion, 299).

indicated that the molar ratios of syringyl to guaiacyl moieties (S/V), as measured by that of syringaldehyde to vanillin, varied among organs (Table 1). The S/V ratios of stem and fruit coat were 0.79 and 0.85, respectively, and were higher than those in other organs (Table 1). The S/V ratios of kernel and squeezed kernel could not be calculated, because product yields were trace.

Next, the lignin content of each organ of *J. curcas* was

Table 1. Lignin contents

Organs	Lignin content (%)	S/V ratio
Stem	15.90±0.24	0.79±0.05
Leaf	9.14±0.32	0.08±0.02
Fruit coat	14.32±0.91	0.85±0.02
Seed	35.92±3.19	0.28±0.01
Seed coat	49.42±1.11	0.28±0.01
Kernel	1.93±0.30	ND
Seed cake	41.38±1.03	0.26±0.00
Squeezed kernel	2.19±0.14	ND
Hermaphrodite flower	3.64±0.03	0.09±0.02
Male flower	4.44±0.23	0.20±0.00

S/V: syringaldehyde/vanillin. ND: not determined because of low yields of products.

determined using the thioglycolic acid method (Suzuki et al. 2009; Table 1). The lignin contents of the stem of the six-month-old plant and the secondary xylem of one-year-old stem were 15.90 and 19.88%, respectively, lower than the previously reported value of 24.11% (Valthanomsat and Apiwatanapiwat 2009). The difference might be due to maturity of the stems, although the effects of growth conditions or genetic background cannot be eliminated. The young, low-lignin stems can be used for enzymatic saccharification, because in general lignins impede access of hydrolyzing enzymes to lignocellulosic polysaccharides. The seed had high lignin content of 35.92%. The lignin contents of seed coat and kernel were 49.42% and 1.93%, respectively. The very high lignin content of the seed coat was consistent with previous reports; Makkar et al. determined acid detergent lignin in seed coats and kernels of *J. curcas* from Nicaragua, Nigeria, and Mexico, and showed that the lignin contents were 45.1–47.5% in the seed coats and ca. 0.2% in the kernels (Makkar et al. 1998; Makkar and Becker 2009). In general, large amounts of lignins accumulate in the seed coat. The seed coat of *Carthamus tinctorius* was strongly stained red by phloroglucinol-hydrochloric acid reaction, while the kernel was not stained (Sakakibara et al. 2007). The lignin contents of seed coat were 31.3% in cotton (Al-Masri 1999), 31.4% in sunflower (Haykiri-Acma and Yaman 2009), and 36.9% in cherry (Duman et al. 2011). These lignin contents were equal to or higher than those of conifer woods (24–33%; Sarkanen and Hergert 1971); that of the *J. curcas* seed coat was the highest among these seed coats. The seed cake obtained by squeezing the seeds had high lignin content (Table 1), which can be exploited to generate electric energy by burning. Thus, the seed cake and oil obtained by squeezing the seeds could be used for electric energy generation and biodiesel production, respectively. The lignin contents of leaf and fruit coat were 9.14% and 14.32%, respectively, similar to previous reports [leaf, 11.1% (Ruíz-Valdiviezo et al. 2010); fruit

coat, 12% (Jingura et al. 2010)]. The hermaphrodite and male flowers had low lignin levels, 3.64 and 4.44%. Flowers are not highly-lignified organs, but lignification can occur. For example, anther walls of *Arabidopsis thaliana* lignified for anther dehiscence (Mitsuda et al. 2005). The variation in lignin contents among the organs was roughly in accordance with the differences in yields of nitrobenzene oxidation and thioacidolysis products.

In conclusion, we characterized the lignins in several organs of *J. curcas*. This study provides basic data for making pulp and paper, particle boards, and wood pellets from *J. curcas* wood. In addition, these data can be used to calculate the energy of combustion.

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