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Synthesis, characterization, and antifungal activity of novel inulin derivatives with chlorinated benzene

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ABSTRACT

A group of novel inulin derivatives containing benzene or chlorinated benzene were synthesized by reaction of chloracetyl inulin (**CAIL**) with the Schiff bases of 4-amino-pyridine, including (2-pyridyl)acetyl inulin chloride (**PAIL**), 2-[4-(2-chlorobenzylideneamino)-pyridyl]acetyl inulin chloride (**2CPAIL**), 2-[4-(4-chlorobenzylideneamino)-pyridyl]acetyl inulin chloride (**4CPAIL**), and 2-[4-(2,4-dichlorobenzylideneamino)-pyridyl]acetyl inulin chloride (**2,4DCPAIL**). Their antifungal activity against three kinds of phytopathogens was estimated by hypha measurement *in vitro*. Of all the synthesized chitosan derivatives, **2,4DCPAIL** inhibited the growth of the tested phytopathogens with inhibitory indices of 67%, 47%, and 43% against *Colletotrichum lagenarium* (Pass) Ell.et halst, *Phomopsis asparagi* (Sacc.) Bubak and *Fusarium oxysporum* (schl.) F.sp. niveum (*F. oxysporum*) respectively at 1.0 mg/mL. The results indicate that all the inulin derivatives have better antifungal activity than inulin, and the inhibitory index is affected by the chlorine atom grafted to the inulin derivatives.

1. Introduction

As a kind of abundant natural polysaccharide, inulin is mainly extracted from such low-requirement crops as Helianthus tuberosus, chicory, and yacon. Inulin consists primarily of β-fructosyl fructose units – always presented in furanose form – usually with a glucopyranose unit reducing end (GFn) (Rogge & Stevens, 2004). Inulin has unique range of molecular weight with the degree of polymerization (DP) varies from 2 to 70, which is rare as a kind of polysaccharide. This polysaccharide has exhibited some interesting properties such as beneficial nutritional attributes for human health, moderate average degree of polymerization and readiness of being obtained (Beylot, 2006; Causey, Feirtag, Gallaher, Tungland, & Slavin, 2000). As a kind of source of renewable, biodegradable and environmentally benign polysaccharide, inulin is a promising candidate to meet the continuously increasing demand for biodegradable and environmentally benign polymeric material. This structural singularity raises its interesting properties like beneficial nutritional attributes, which in turn excites the chemical modification of inulin in recent years (Beylot, 2006; Habibi & Dufresne, 2008). We have reported the antioxidant activity and antifungal activity of inulin derivatives (Ren, Liu, Dong, & Guo, 2011; Ren et al., 2012), which proved the chemical modification could improve the bioactivity of inulin.

Many reports have demonstrated that aromatic nucleus Schiff bases had good bioactivities (Karthikeyan et al., 2006). It is also reported that salicylaldehyde derivatives, with one or more halo-atoms in the aromatic ring, showed a variety of biological activities (Li, Guo, & Jiang, 2010). Meanwhile, as antifungal group, chlorinated benzenes are used in many fungicides such as pentachloronitrobenzene and chlorothalonil which have five or four chlorine atoms respectively (Sha, 1994). Chemical fungicides with chlorine atoms have good fungicidal activity and used widely years ago, but their toxicities and residues give rise to serious problems for environment and human health. When these groups are grafted onto inulin, they should be released slowly and may meet the requirements of environmental safety.

In this paper, we reported the synthesis and antifungal properties of a group of inulin derivatives with benzene or chlorinated benzene as substituent including **PAIL**, **2CPAIL**, **4CPAIL**, and **2,4DCPAIL**. The chloracetyl inulin (**CAIL**) was first synthesized by reaction between the C-6 hydroxyl of inulin and chloracetyl chloride. **CAIL** was an excellent intermediate of the project as the chlorine of **CAIL** can easily attack pyridine to give *N*-alkypyridinium salts (Li et al., 2013; Sajomsang, 2010). Subsequently the benzene or chlorinated benzene groups were introduced into inulin through the reaction between Schiff bases of 4-amino-pyridine and **CAIL**.

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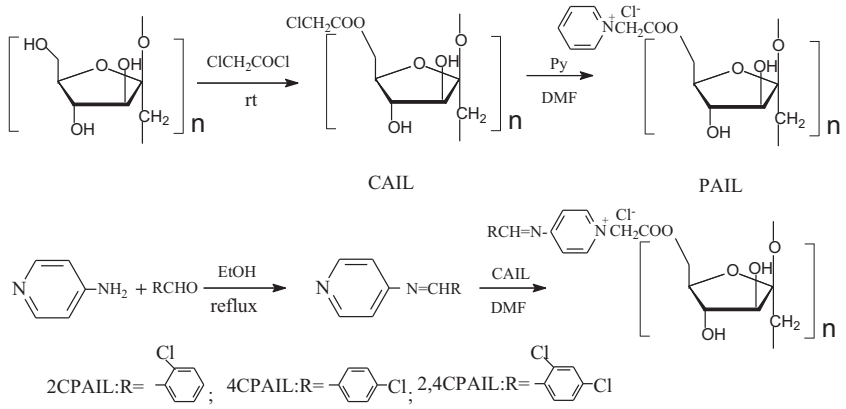

Scheme 1. Synthetic routes for inulin derivatives.

The aimed inulin derivatives designed in this way were expected to have advantageous features such as high antifungal activity and good water solubility. Apart from these advantages, the reaction generates Schiff bases functional groups, which is also helpful for the constructions of bioactive inulin derivatives (Guo, Ren, Dong, Wang, & Li, 2013). The chemical structures of the derivatives were characterized by FT-IR and ^{1}H NMR.

Plant pathogenic fungi negatively affect a large number of important vegetables and fruits. The losses caused by plant pathogenic fungi have attracted wide attention throughout the world. For example, *Phomopsis asparagi* can cause stem blight of asparagus and subsequent yield loss; the premature plant death caused by *Colletotrichum Lagenarium* can lead to a yield loss of 6–48% (Chen & Dai, 2012; Elena, 2006). Therefore, three common plants-threatening fungi, *C. Lagenarium*, *P. asparagi, and Fusarium oxysporum*, were selected to evaluate the antifungal properties of the derivatives by hypha measurement *in vitro*. Control of these plants-threatening fungi could benefit the production of related vegetables and fruits.

2. Experimental

2.1. Materials and analytical methods

Inulin was purchased from E. Merck (Darmstadt, Germany). 2-Chlorobenzaldehyde, 4-chlorobenzaldehyde, and 2,4-dichlorobenzaldehyde were purchased from the Sigma–Aldrich Chemical Corp. The other reagents are all of analytical grade and used without further purification. FT-IR spectra were recorded on a Jasco-4100 Fourier Transform Infrared Spectrometer (Japan, provided by JASCO Co., Ltd Shanghai, China) with KBr disks. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were measured with a Bruker AVIII-500 Spectrometer (Switzerland, provided by Bruker Tech. and Serv. Co., Ltd. Beijing, China.). Three plants-threatening fungi *C. lagenarium* (Pass) Ell.et halst, *P. asparagi* (Sacc.) Bubak and *F. oxysporum* (schl.) F.sp. niveum (*F. oxysporum*) were provided by Qingdao Academy of Agricultural Sciences.

2.2. The synthesis of the Schiff bases of 4-amino-pyridine

As shown in Scheme 1, a solution of 0.01 mol aldehydes (2-chlorobenzaldehyde, 4-chlorobenzaldehyde or 2,4-dichlorobenzaldehyde) and 0.01 mol 4-amino-pyridine were refluxed in 30 mL ethanol for 4 h. Then the solvent was concentrated under reduced pressure. The crude products were purified by crystallization from ethanol. (Kaboudin, Afsharinezhad, & Mohammad, 2010).

2.3. The synthesis of chloracetyl inulin

Chloracetyl inulin (**CAIL**) was synthesized as follows (Li, Guo, & Jiang, 2010). 1.61 g inulin was dissolved in 100 mL H_2O at room temperature (r.t.), and 0.02 mol chloracetyl chloride was added drop wise. After stirring for 12 h at r.t., the solution was concentrated under reduced pressure. The concentrated solution was precipitated by the addition of excess acetone and the precipitate was filtered. The products were washed with ether dried at 60 °C for 6 h.

2.4. The synthesis of inulin derivatives

A solution of 0.3 g **CAIL** and 0.5 mL pyridine or the Schiff bases of 4-amino-pyridine in 20 mL *N*, *N*-dimethylformamide (DMF) was stirred for 24 h at 60 °C. The solution was precipitated with excess acetone and the precipitate was filtered. The unreacted pyridine, amine, and other outgrowth were extracted in a Soxhlet apparatus with ethanol for two days. The inulin derivatives were obtained after drying at 60 °C for 6 h (Li, Guo & Jiang, 2010).

2.5. Antifungal assays

Antifungal assays were performed by following the plate growth rate method (Jasso de Rodríguez, Hernández-Castillo, Rodríguez-García, & Angulo-Sánchez, 2005; Li, Guo & Jiang, 2010). Briefly, the compounds were dissolved in distilled water at a concentration of 5.0 mg/mL. Then, each sample (inulin, **CAIL** and inulin derivatives) solution was added to sterilized potato dextrose agar to give a final concentration of 0.1, 0.5, and 1.0 mg/mL. After the mixture was cooled in the plate (6.0 cm diameter), the mycelium of fungi of a 5.0 mm diameter was transferred to the test plate and incubated at 27 °C. When the mycelium of fungi reached the edges of the control plate (without the presence of samples), the antifungal index was calculated as follows:

$$\text{Antifungal index}\,(\%) = 1 - \frac{D_a}{D_b} \times 100$$

where D_a is the diameter of the growth zone in the test plates and D_b is the diameter of the growth zone in the control plate. Each experiment was performed three times, and the data were shown with mean ± SD. The Scheffe method was used to evaluate the differences in antifungal index in the antifungal tests. Results with $P < 0.05$ were considered statistically significant (Jasso de Rodríguez et al., 2005).