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SYNTHESIS OF NOVEL PIMARICIN INULIN CONJUGATE

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ABSTRACT

Solubleinulin pimaricin conjugate have been synthesized by coupling of aldehyde-*functionalized* polysaccharide inulin with awater-insoluble tetraene macrocyclic membrane-active antifungal antibiotic pimaricin. The synthesized product was soluble in water and characterized by UV-VIS and ^{1}HNMR spectroscopic data.

INTRODUCTION

Reactions of covalent coupling of various aldehyde-*functionalized* polysaccharides with biological active compounds has successfully been applied for decades. [1]. The polyaldehydo polymers obtained by periodate oxidation of polysaccharides, *via* reactive -CHO groups can facilely bond amine-containing drugs, such as polyene antibiotics, through the Schiff base linkages thus enhancing the water solubility of insoluble drugs and therefore their availability and functionality.

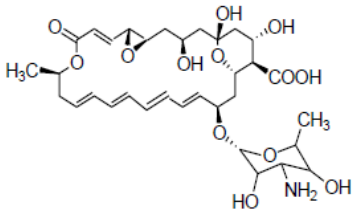

Figure 1.
Structure of pimaricin

Pimaricin is a polyene macrolide antibiotic produced by *Streptomyces natalensis*. Its consists of large lactone ring which is linked to a mycosamine moiety, deoxyamino-sugar, by a glycosidic bond (Fig.1.) Pimaricin is a potent antifungal compound which specifically inhibits the growth of mold and yeast, and mostly used in food industry as a natural preservative and medicine[2]. However, insolubility in water limits its wider application.

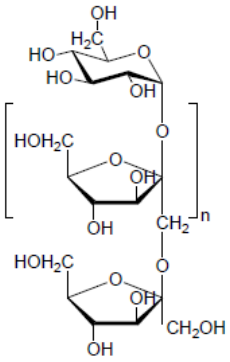

Figure 2. Inulin

Inulin is a soluble polysaccharide belonging to a heterogeneous group of carbohydrates known as fructans. This polymer consist of chain-terminating glucosyl moieties on nonreducing end, and a repetitive fructosyl moiety which are linked by β-(2,1) glycosidic bonds (Fig. 2). Due to its excellent features: non-toxicity, water solubility and non-digestibility inulin and its partial hydrolysis products, oligofructose, are used in many areas: in the food industry, pharmacy, medicine [3]. Since the application of the pimaricin rather limited because of its insolubility in aqueous media the goal of the present work was to synthetize conjugate of this antibiotic by covalent coupling with aldehyde-*functionalized* inulin in order to increase solubility of the antifungal compound. Water soluble conjugate was characterized by UV-Vis and ^{1}HNMR data.

EXPERIMENTAL

Oxidation of inulin to polyaldehyde derivative was performed following a procedure reported by Tabandeh and Aminlari [4]. Aldehyde functionalized inulin (40 mg) and pimaricin (40 mg) were coupled in borate buffer (pH 8.0) in the dark, with continuos stirring, at 30 °C, during 24 h. Resulting conjugate was precipitated by 96% ethanol (three volumes). After centrifugation, procedure of disolving in water (5 mL) and precipitation with ethanol was repeated for three times. The conjugate was additionallypurified by *gel filtration* on *Sephadex* G-10 using distilled *water* as an *eluent*and lyophilized (Christ Alpha 2-4 LD plus). ^{1}H NMR spectra were recorded on a Varian Gemini 2000 (200 MHz). UV-VIS spectra were measured on Shimadzu UV-1280 spectrophotometer.

RESULTS AND DISCUSSION

Synthesis of periodate oxidized inulin-pimaricin conjugates was achieved by coupling reaction between aldehyde groups of oxidized polysaccharide and amine group of the antibiotic. Coupling reaction was monitored by UV-Vis and ^{1}HNMR spectroscopy. It is known that native and oxidized glycans do not have absorptions in UV-Vis range of 250-400 nm [5].However, after coupling reaction with tetraene macrolide, spectrum of synthesized conjugate (Fig. 3) shows characteristic absorptions (λ max291, 304, and 319 nm) slightly red shifted (up to 3 nm) inrelation to pimaricin, as shown in Figure 3[6]. This is probably *due* to interchain interactions on the electronic

structure of synthesized conjugate.Based on this, it can be concluded that aldehyde-*functionalized* inulin was successfully coupled with -NH_2 group of antibiotic.

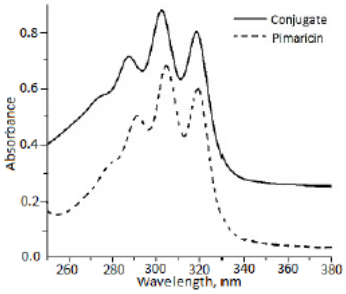

Figure 3. UV spectra of pimaricin and inulin-pimaricin conjugate

^{1}H NMR spectrum of synthesized conjugate (Fig. 4) showed characteristic peaks of ^{1}H protons of oxidized inulin, as well as peaks of pimaricin. It can be concluded that some signals related to inulin: H-1 anomeric protons of the glucose residues (5.4–5.0 ppm), protons from the –OH groups of the fructosyl residues (4.9-4.2) and signals of the carbon protons of fructosyl units (3.7-2.5) are overlapped with some peaks characteristic of the pimaricin: polyol segment and part of signals of hemiketal six-membered ring (4.50–3.0) [7,8]. Except these overlapping signals, the part of the spectrum related to the polyene region of the antibiotic, and some signals of monosaccharide residues of inulin, are specificallyevidenced.

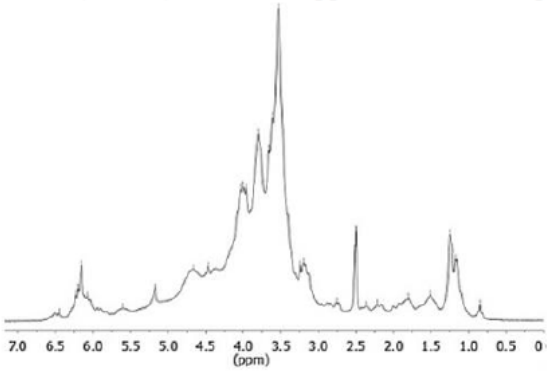

Figure 4. ^{1}HNMR spectrum ofinulin-pimaricin conjugate

In the ^{1}HNMR spectrum of conjugate chemical shifts characteristic for the polyene regions (5.50–6.51 ppm) of pimaricin was clearly distinguished, as well as methylene region (2.50–1.40) and methyl groups of this antibiotic (1.24–0.85) [8].

CONCLUSION

The macromolecular conjugate was synthesized by coupling reaction between aldehyde-*functionalized* polysaccharide inulin and *tetraene* macrolide antibiotic pimaricin. The o*btained product* was characterized by UV-Vis and ^{1}HNMR data. This novel product potentiallycouldhave a great usein food industry as a resultof improved drug solubility in water.

Acknowledgement

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