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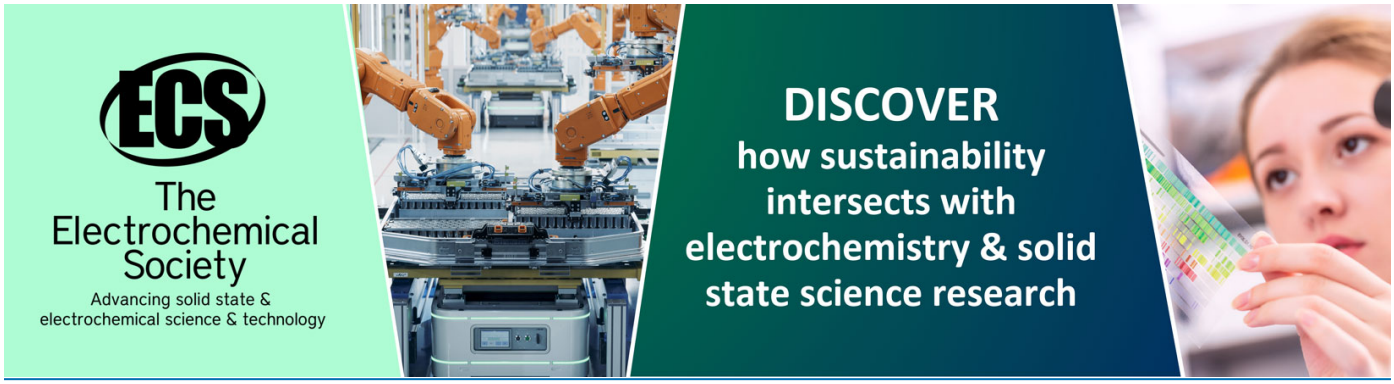

Synthesis of Sulfonated burdock fructooligosaccharide (BFO)

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Abstract. Burdock Fructooligosaccharide (BFO) were sulfated using SO3-Py complex. The maximal degree of sulfonation (DSsulf) was 1.56, which were obtained by varying reaction factor such molar ratio of SO3-Py to fructofuranans unit (FU). The FT-IR, 1H NMR and 13C NMR spectra showed the introduction of sulfate group, and the reaction occurred at C-6, C-4 and C-3 in the fructofuranans unit of BFO. The molecular weight estimated by HPGPC were 6104.7-11003.3 g/ mol for S-BFO (DS sulf=1.2).

1. Introduction

Burdock fructooligosaccharide (BFO) is a fructosan oligomer isolated from the root tissue of *Arctium lappa*, which is composed of a linear chain of twelve β-2, 1 linked fructofuranans residues with one terminal α-1, 2 linked glucopyranose (GF_{12}) [1]. The BFO has the bioactivities of defense and protection both cucumbers and tomatoes against several diseases caused by pathogens [2-6]. The resource of BFO is abundant: burdock is commonly planted as a popular vegetable and traditional medicine in China, Japan and many other Asian countries; the amount of BFO in the air-dried root tissue is about 17.0%, by the simple water extraction and ethanol precipitation, the distribution of BFO was over 90% and the yield was 85% [1]. The BFO is one of the most intensively studied fructooligosaccharide-inulin (FOSI) [7], synthesis of new BFO derivatives with improving bioactivity, good water-solubility, or other new properties is meaningful for widening its application range. But until now, synthesis of new derivatives of BFO and other fructooligosaccharide (FOS) from burdock has not been reported.

BFO is a of kind inulin with the degree of polymerization (DP) 13, which structure is shown in Fig. 1. We proposed to synthesis sulfated BFO (S-BFO) using SO_3-Py method, and the S-BFO was respected to be more bioactivity and better solubility in water than that of BFO. This study addressed the sulfation of BFO, and the influence of molar ratio: SO_3-Py to fructofuranans on the degrees of substitution (DS) were investigated. The characterizations of S-BFO were performed by FT-IR, ^{1}H NMR, ^{13}C NMR and high-performance gel permeation chromatography (HPGPC). Potential applications in material fields and biological activities of S-BFO in biological chemsitry are investigating in our laboratory.

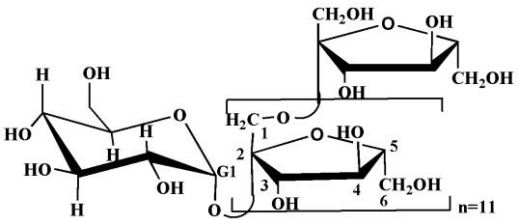

Figure 1. The structure of BFO

2. Experimental

2.1. Material and Methods

Burdock root was obtained from the local market and stored at -20℃ until used. BFO was obtained according to the previously described method and which average molecular weight was 2134 g/mol [1]. *N*, *N*-dimethylformamide (DMF), SO_3-Py were bought from Shanghai Yue Ling Chemical Company. Dialysis membranes (MWCO 3600) were purchased from Sigma. The DMF is a chromatographically pure grade and the other chemicals and reagents were of analytical grade.

Nuclear magnetic resonance spectra were obtained on Bruker Avance 600 MHz and Bruker Avance 300 MHz spectrometer, using TMS as internal standard substance. Elemental analysis of sulfur was performed using Carlo Erba EA 1108 CHNS analyzer. Infrared spectra were recorded on a Nicolet NEXUS 670 FT-IR spectrometer, and the samples were prepared as KBr pellets.

The degree of sulfonation (DS_{sulf}) designates the average number of sulfonyl groups on each sugar-residue, which was determined by elemental analysis method. The DS_{sulf} was calculated using Eq. 1,

$$DSsulf = (S\% / C\%) / (32 / 72) \quad (1)$$

where S% and C% are the percentage of sulfur and carbon on dry basis respectively; 32 is the atomic weight of sulfur and 72 is the total molecular weight of carbon on fructose or glucose unit.

2.2. The sulfation reaction of BFO

The BFO (0.326 g) was dissolved in DMF (10 mL) and stirred for 10 min at room temperature. Subsequently, a specific amount of SO_3-Py was added. The mixture was stirred for 2 hours at 40℃, then 10 mL of ice water was added to terminate the reaction. The mixture was cooled to room temperature and the pH was adjusted to 8-9 with 15% NaOH solution. The collecting liquid was concentrated, dialyzed for 72h and lyophilized to give the product of S-BFO.

2.3. Assessment of homogeneity and molecular weight

The homogeneity and molecular weight of the S-BFO were determined by high-performance gel-permeation chromatography (HPGPC) (Agilent technologies- 1100, USA) equipped with a TSK-gel G2000PW column (7.5 mm × 300 mm, column temperature 30 ℃) and Refractive Index Detector (RID, detecting temperature 35 ℃). A small amount of sample solution (15 μ L) was performed in each run at a flow rate of 0.5 mL/min with water as their mobile phase. The column was calibrated with T-series dextran (T-10, 40, 70, 500, 2000) as standards, and the molecular weight of S-BFO fractions was estimated by reference to the calibration curve.

3. Result and Discussion

3.1. Synthesis

In the sulfation reaction of BFO, hydrolysis or degradation of BFO is probably happened in the strong acidity of SO_3-Py and high reaction temperature, and the SO_3-Py is easy to hydrolyze in the presence of water. Then chromatographically pure grade of DMF and low reaction temperature were used to

avoid the hydrolysis or degradation of BFO and the hydrolyze of SO_3-Py in the reaction. Then at the reaction temperature 40℃, reaction time 2 hours, a series of sulfated BFO with different DS_{sulf} were obtained by varying the SO_3-Py complex /FU molar ratio. As Fig. 2 shown, the maximal DS_{sulf}=1.56 was obtained with the reaction condition: SO_3-Py complex/ FU molar ratio: 6/1, reaction time 2 h and reaction temperature 40℃. In the experimental, the yield of the S-BFO is in range of 85% to 95%.

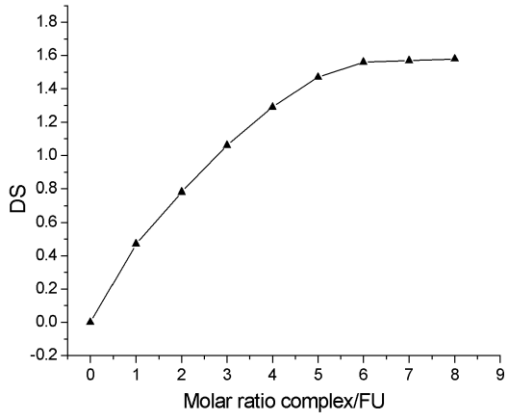

Figure 2. Influence of Molar ratio: SO3-Py /fructofuranans unit (FU) on the DSsulf in sulfation reaction of BFO. Reaction time: 2h, reaction temperature: 40 ℃.

3.2. FT-IR spectra of S-BFO

Fig. 3 showed the FT-IR spectra of S-BFO with different DS. In Fig. 3, by comparing IR spectra of S-BFO with IR spectra of BFO, the new strong adsorption at 1258 cm^{-1} and 1057 cm^{-1} were attributed to asymmetrical and symmetrical S=O stretching vibration respectively, and the new absorption at 813 cm^{-1} was attributed to the C-O-S stretching vibration assigned to a C-O-SO_3 group and the C-H blending vibration in furan ring of BFO was covered. Moreover, the absorption band of SO_3 group were enhanced accompanied with DS_{sulf} increasing in Fig 3. These results above indicate the sulfation reactivity of BFO.

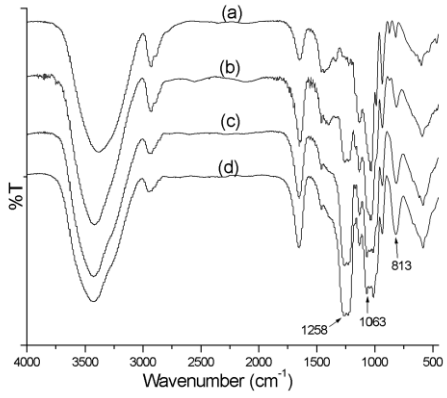

Figure 3. FT-IR spectra of BFO and sulfated BFO with different DS sulf: a: BFO, b: DS sulf=0.2, c: DS sulf=0.7, d: DS sulf=1.2;

3.3. ^{1}H NMR and ^{13}C NMR spectra of S-BFO

The ^{1}H NMR spectra of BFO and S-BFO (DS_{sulf}=1.2) recorded on Bruker Avance 300 MHz in D_2O. The ^{13}C NMR spectra of BFO and S-BFO (DS_{sulf}=1.2) were recorded on Bruker Avance 600 MHz in D_2O. Refering to the data on inulin and BFO reported early [1, 8-9], detailed assignments of the

signals are listed in Table 1, and the C atom numbers on fructose residues were marked as Fig. 1. In table 1, some bands of protons of BFO are about 0.4 ppm downfield shift caused by introduction of sulfate groups, which was in accord with the literature report: O-sulfation caused downfield shifts of protons bound to the O-sulfated carbon atoms by approximate 0.4-0.7 ppm for carbohydrate [10-11]. The ^{13}C NMR data indiated that the reaction position of sulfation are at the C-6, C-4 and C-3 of the fructofuranans. As a whole, the ^{1}H NMR and ^{13}C NMR data confirmed the presence of O-sulfate groups.

Table 1. 1H and 13C NMR chemical shifts (ppm) for residues of BFO and S-BFO (DSsulf=1.2)

Compound		Fructose: →1)-β-D-Fruf-(2→									Glucose: α-D-Glop-(1→
		1	2	3	4	5	6	3'	4'	6'	G1
BFO	H	3.56		4.12	3.96	3.78	3.70				5.30
	C	60.3	103.0,	76.8	74.1	81.0	62.0				92.3
S-BFO (DS=1.2)	H	3.67		4.26	4.22	4.02	3.88,	4.48	4.37	4.12	n.d.
	C	60.2	104.5	77.4	74.3	80.8	62.0	78.3	76.2	68.2	92.8

[a]n.d.=not determined.

3.4. Molecular weights of S-BFO

Besides DS, the molecular weight of polysaccharide is another important parameter influencing its properties. As we know, degradation of the polysaccharide during the sulfation was a common problems [12]. Then the molecular weight of S-BFO (DS_{sulf}=1.2) were measured by HPGPC method, and the HPGPC profiles of them are shown in Fig. 4. The molecular weight of S-BFO (DS_{sulf}=1.2) was obtained as 6104.7-11003.3 g/mol. The results indicated that no degraded occurred in the sulfated process of BFO under this mild reaction conditions. Furthermore, wide molecular weight distribution and high molecular weight as well as 6104.7-11003.3 g/mol for S-BFO (DS_{sulf}=1.2) indicated that crosslinking of BFO molecules may happened by reaction with SO_3-Py.

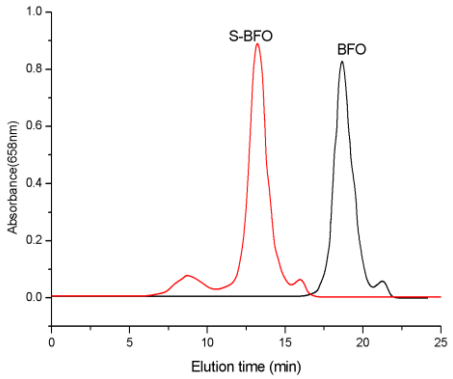

Figure 4. High-performance gel permeation chromatograph (HPGPC) profiles of BFO and S-BFO (DSsulf =1.2).

4. Conclusion

In summary, Sulfated derivatives of BFO were synthesized using SO_3-Py complex, and the maximal degree of sulfonation (DS_{sulf}) were 1.56. The FT-IR spectra, ^{1}H NMR and ^{13}C NMR of the products shown the characteristic of S-BFO, and the reaction position of sulfation are at the C-6, C-4 and C-3 of the fructofuranans. The molecular weight of the S-BFO (DS_{sulf}=1.2) determined by HPGPC are 6104.7-11003.3 g/mol, which indicated that no degradation of BFO occurred in the reaction process.

By introduction of sulfate ester groups, the water-solubility of BFO was increased greatly, we have observed that several mg of S-BFO (DS=1.2) could be dissolved in 1 mL of a water at ambient temperatures in the experiment. We supposed that introduction of sulfate ester groups resulted in the conformation changed of BFO and more hydroxyl groups exposed, and its molecular polarity was enlarged significantly also. Those are benifite to investigate new applications in material and biological chemsitry fields. The biological activities of S-BFO in biological chemsitry and potential applications in material and fields is investigating in our laboratory.

Acknowledgments
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