1D-NMR SPECTROSCOPY OF THE POLYSACCHARIDE STARCH FROM *Plectranthus esculentus.* A POTENTIAL EXCIPIENT

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Abstract: *Plectranthus esculentus* (Lamiaceae)tubers in Jos, Nigeria produced a white starch. This starch was characterized by the application of Fourier Transform Infrared (FTIR) and 1-Dimensional (ID) Nuclear Magnetic Resonances (NMR) spectroscopy. Application of FTIR and ID-NMR spectroscopy to the original starch from *P.esculentus* tubers in combination with chemical data, led to confirm that the structure of the original polysaccharide contains α-D-glucose linked at 1 → 4. It was demonstrated that ID-NMR spectroscopy is a good tool for structural information of complex hetero-polysaccharides.
Keywords: *Plectranthus esculentus*, polysaccharide, FTIR, ID-NMR, spectroscopy

Introduction

In recent years, the development and utilization of new polysaccharides isolated from natural sources have attracted increasing attention in biochemistry, pharmaceuticals and food chemistry due to their sustainability, biodegradability and biosafety (Dodi *et al.,* 2011; Cristina Freire *et al.,* 2009). In the year 2000, the world starch market was estimated to be 48.5 million tons, including native and modified starches. The value of the output is worth €15 billion per year. Worldwide, the main sources of starch are Maize (82%), Wheat (8%), Potatoes (5%) and cassava (5%) (Le corre *et al.,* 2010).Starch consist of two structural components, the amylase, which is essentially a linear polymer in which glucose residues are $\propto -D-(1-4)$ linked typically constituting 15% to 20% of starch and amylopectin which is a larger branched molecule with $\propto -D-(1-4)$ and $\propto -D-(1-6)$ linkages and is a major component of starch (Sajilata *et al.,* 2006). It is obvious that the need for new starches from local sources will continue to increase especially as this biopolymer finds application in other industries including medicine and pharmacy.

The *Plectranthus* belong to the mint family Lamiaceaeto which aromatic plants belong. It is a perennial herb which is sparsely branched and grown up to 2 m in height. The tubers are cultivated for food in Africa, particularly in the Northern parts of Nigeria around Adamawa, Bauchi, Niger, Kaduna and most importantly Jos, Plateau State (Kyemsu, 1994; Allemann and Hammes, 2003). The leaves are used in the treatment of various ailments such as respiratory and digestive problems (Kyemsu, 1994). Nutrient content, binding and disintegrating properties of *P. esculentus* starch in drug formulation have been reported (Kemas *et al.,* 2013; Ochekpe *et al.,* 2013; Temple *et al.,* 1991).

The pharmaceutical significance of the starch from *P. esculentus*have been reported but the structural studies of the starch is not yet ascertained. In order to carry out rational product design and development, it would be desirable to characterize the structure of this polysaccharide and to improve the understanding of structure and property relationships. It is expected that the starch from *P. esculentus* should show structural properties common to known starches. Therefore, this work deals with the structural studies of the polysaccharide starch extracted from *P. esculentus* tubers by the application of FTIR and 1D-NMR spectroscopy.

Materials and Methods

Extraction of *P. esculentus* Starch *P. esculentus* potatoes tubers were obtained from Angwa-Rukuba market, Jos. Nigeria. Washed, peeled, and trimmed to remove defective parts. The tubers were then sliced, diced, and blended with distilled water in a food blender. The mixture was sieved through an 80-mesh screen, and the retained solid was exhaustively rinsed on the sieve with distilled water. The filtrate was allowed to stand overnight at 15^0C, the precipitate was collected, and the supernatant was discarded. Resuspension and sedimentation operations were repeated until white starch was obtained. The starch was dried at 50^0C for 6 h. Finally, the dried potato flir was ground and passed through a 100-mesh sieve. The starch was kept in a tight light-resistant container.

Fourier transform infrared (FTIR) and 1D-NMR spectroscopy

The FTIR spectrum of the sample was recorded in an FTIR spectrometer (Nicolet Magna 4R 560. MN USA) using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr ^{1}H NMR, ^{13}C-NMR, ^{13}C-DEPT and Solid State NMR of hydrolysed *P. esculentus* starch were recorded in an NMR (600 MHz) spectrometer (Agilent technologies, America). The sample (10 mg) was dissolved in 700 µL at 70^0C with continuous stirring for 6 h followed by sonication for 10 min. The sample was centrifuged and transferred to a 5 mm NMR tube. Chemical shifts were reported in ppm relative to an internal standard TMSP (Tetramethyl-silanepropoinic acid). Peak integra were performed using Agilent software, America. Dried sample was packed carefully inside the NMR rotor for solid state NMR analysis.

Plate **1:** *P. esculentus* tubers

Results and Discussion

FTIR spectroscopy

In the FTIR spectrum of the native starch (Fig. 1), there are several discernible absorbencies at 1159 and 1082 cm^{-1}, which are attributed to C-O bond stretching (Gohen and Wool, 1991). Additional characteristics absorption bond at

992, 929, 861, 765 and 575 cm^{-1} are due to the entire anhydroglucose ring stretching variations. The extreme broad band between 3000-3500 cm^{1} and the peak at 2,950 cm^{-1} correspond to OH and CH stretchings, respectively, while the peaks at 1647 cm^{-1} correspond to (OH) bending (Mano *et al.*, 2003).

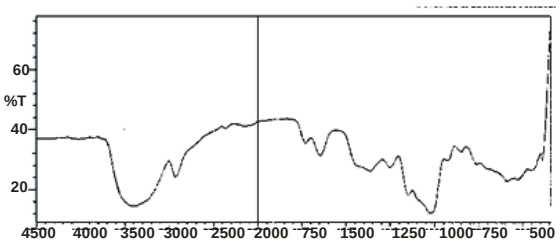

Fig. 1: FTIR of *P. esculentus* starch

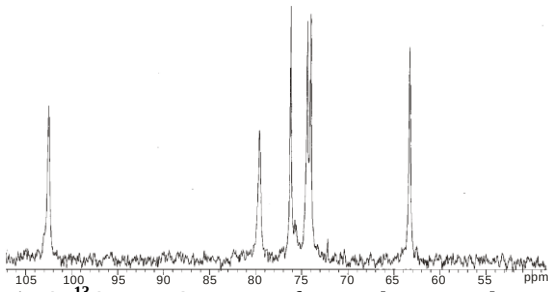

Fig. 2: ^{13}C NMR Spectrum of *P. esculentus*starch

NMR spectroscopy

One-dimensional NMR techniques that are based on highly predictable chemical shifts for specific molecular environment have been used extensively for the determination of carbohydrate structure (Duus *et al.*, 2000; Bock and Thogersen, 1982). Despite thefact that most resonances on ^{1}H spectra are clustered between 3.4 ppm and 4.0 ppm, ^{1}H spectra of polysaccharides do contain some well-resolved signals including those of anomeric proton (4.4-5.5 ppm), acetyl (2.0-2.1 ppm) and methyl (1.2 ppm) (Bock and Thogerson, 1982). The ^{1}H and ^{13}C NMR spectra of *P. esculentus* starch are shown in Figs. 2 and 4, respectively. The ^{1}H NMR spectrum (Fig. 4) crowded in a narrow region between 3 to 5 ppm is typical of polysaccharides (Cheng and Thomas, 2012; Bubb, 2003; Mirau, 2004). The signals between 3.5 and 4.2 ppm can be assigned to non anomeric protons (H-2 to H-6) while the signal around 5.3 ppm arise from anomeric proton (H-1). Which is compatible with the expected conformation of $\propto -anomer$ since $\propto -anomeric$ proton of polysaccharides are in the range of 5 to 6 ppm (Cheng and Thomas, 2012; Mirau, 2004). ^{1}H NMR spectra tend to have overlapping signals in the 3.2 to 4.2 ppm region and coupling information is difficult to assign. However, the anomeric proton signal 5.3 ppm corresponds to $\propto -D-glucose$ residue (Bubb, 2003; Mirau, 2004; Cheng *et al.*, 2001). The resonances of the hydrogen atoms H-2 to H-6 were well resolved as 3.63, 3.94, 3.62, 3.82 and 3.88 ppm, respectively (Table 1). Although ^{13}C NMR spectra has much weaker signal, it has significant advantages over ^{1}H NMR spectra in the analysis of polysaccharides because the chemical shift are spread out over a broader range (0 – 200 ppm) (Mano *et al.*, 2003). In ^{13}C spectra, signals from anomeric carbons appear in the 90-105 ppm region while the non-anomeric carbons are between 60 and 85 ppm (Cheng *et al.*, 2001). The anomeric C-1 carbon are the most diagnostic; thus from C-1 alone, one can often determine the different types of sequence present and their relative proportions. The resonance of C-2 to C-5 can be found at 65-78 ppm. The primary OH (C-6 for pyranose) resonates at 60-70 ppm (Cheng and Thomas, 2012).

Table 1: ^{1}H and ^{13}C-NMR data of the Sugar residue of *P. esculentus*starch in D_2O at 80^0C reference to TMSP in ppm

	C-1	C-2	C-3	C-4	C-5	C-6
α–D–glucose	**H-1**	**H-2**	**H-3**	**H-4**	**H-5**	**H-6**
	102.6	74.2	75.8	81.3	74.21	62.5
	5.35	3.63	3.94	3.62	3.82	3.88

The carbon anomeric region of ^{13}C NMR of *P. esculentus* srtarch (Fig. 2) showed one major signal which may be attributed to one neutral sugar component of the polysaccharide and was assigned as C-1 of $\propto -D-glucose$ at 102.6 ppm. The spectrum region of the anomeric carbon and the methylene carbon around 62.5 ppm are well depicted (Fig. 2). The resonances of the carbon atoms were well resolved and identified as the resonances of C-2, C-3, C-4, C-5, and C-6 as 74.2, 75.8, 81.3, 74.2 and 62.5 ppm, respectively (Table 1). These facts are almost identical with polysaccharides of other sources (Pollard *et al.*, 2001; Prashanth *et al.*, 2006; Ramesh *et al.*, 2001; Souza *et al.*, 2010).

^{13}C-DEPT (Distortionless enhancement by Polarization Transfer) NMR experiment (Fig. 3) was used to identify the methylene groups' signals which have opposite amplitude to CH and CH_3 carbon. The spectrum showed at a high field one inverted signals (62.5 ppm) which can be attributed to methylene Carbon (C-6) of sugar residue ($\propto -D-glucose$). Inverted signals in DEPT-135^{o} correspond to the free or linked hydroxyl primary groups of the sugar residue ($\propto -D-glucose$) present in the structure. Resonances were assigned with the aid of literature (Chaubey and Kapour, 2001; Tamesh *et al.*, 2001; Vierra *et al.*, 2007; Cunha *et al.*, 2009; Dafauye and Wong, 1986; Tisher *et al.*, 2002).

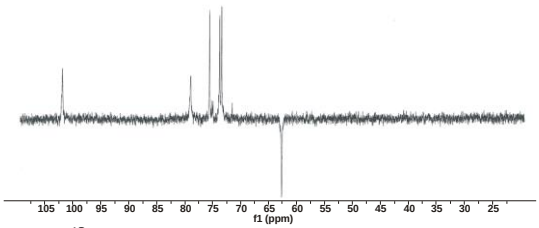

Fig. 3: ^{13}C-DEPT Spectrum of *P. esculentus* starch

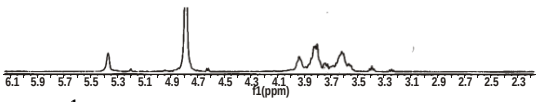

Fig. 4: ^{1}H NMR Spectra of *P. esculentus* starch

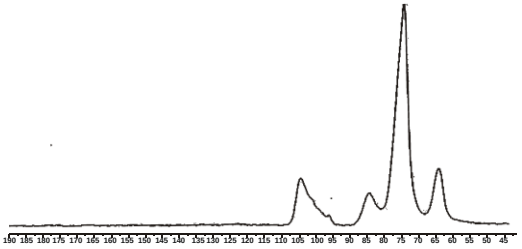

Fig. 5: Solid State ^{13}C NMR of *P. esculentus* starch

Solid State ^{13}C-NMR

The solid state NMR spectrum of the native starch is shown in Fig. 5. The spectrum gave line widths which are typical of natural polysaccharides with broad band signal between 64 and 90 ppm arising from the bulk of the ring, C-OH. The low intensity at about 62.5 ppm is attributable to the $-CH_2OH$ belonging to $\propto -D-glucose$ (Cheng *et al*., 2001). The resonances of the carbon atoms were resolved (Fig. 5) and identified as the resonances of C-1 (102.66), C-2 (74.17), C-3 (75.75), C-4 (81.32), C-5 (74.21) and C-6 (62.5) of $\propto -D-glucose$

Conclusion

The result obtained by FTIR and1D-NMR analysis indicated that the polysaccharide starch from *P. esculentus* contain mainly $\propto -D-glucose$ linked at 1→4. This paper provides substantial ID-NMR structural information for the main polysaccharide from *P. esulentus* tubers.

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