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STRUCTURAL ANALYSIS OF *LILIUM LONGIFLORUM* SPOROPOLLENIN BY ^{13}C NMR SPECTROSCOPY

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Key Word Index—*Lilium longiflorum*; Liliaceae; lily; CP/MAS ^{13}C NMR; exine; sporopollenin; aliphatic polymer; 4-methylmorpholine *N*-oxide.

Abstract—The exine was isolated from lily pollen which had been treated with 4-methylmorpholine *N*-oxide and shown by cross-polarization/magic-angle-spinning ^{13}C NMR to be predominantly an aliphatic polymer.

INTRODUCTION

The outer coat, or exine, of the pollen grain of plants is composed of an unusually resistant polymer known as sporopollenin [1, 2]. Preliminary chemical characterization of intact pollen from a wide variety of plants indicated that sporopollenin is a condensed polymer of carotenoids and carotenoid esters [3–5]. Recent studies on the biosynthesis of sporopollenin, however, have shown that inhibition of carotenoid biosynthesis does not affect sporopollenin formation [6, 7].

Sporopollenin is extremely insoluble in a wide variety of solvents and most palynological studies of this polymer begin by treating the intact pollen with acetic anhydride and sulphuric acid at high temperature [8]. Chemists have used treatments involving hot potassium hydroxide and phosphoric acid solutions [1]. These harsh treatments may bring about changes in the sporopollenin structure [9].

A recent paper has reported the NMR spectra of sporopollenins from five different sources which were isolated by the conventional harsh treatment [10]. The solid state ^{13}C NMR data documented a diversity of molecular structure in each sample. We have obtained ^{13}C CP/MAS NMR data on an exine isolated from the pollen of *Lilium longiflorum* by utilizing a mild treatment which ruptures the pollen grain and enables one to recover both sporoplasts and a very pure sporopollenin fraction [11, 12]. We note both similarities and differences in the spectral data previously published and report that this sporopollenin is primarily an aliphatic polymer.

RESULTS AND DISCUSSION

The ^{13}C NMR spectrum of isolated exine from lily pollen indicates that a large portion of sporopollenin is aliphatic in nature (Fig. 1). The resonance frequencies corresponding to CH_3 and CH_2 groups (δ10–50) comprise *ca* 47% of the carbon in the spectrum. Carbohydrate carbons (δ55–90) contribute another 26% while those in the δ90–150 range (alkene and aromatic carbons plus the anomeric carbohydrate carbon) account for only 16% of the total carbon. The carboxyl carbon region (δ160–190) comprises 10% and contains distinct regions characteristic of ester (δ160–170) and acid (δ170–180) functional groups with the latter the predominant species. Another 2% of the carbon in the exine fraction is of the ketone carbonyl type (δ200–210). The spectrum indicates that this sporopollenin sample is a polymer composed primarily of long chain aliphatic monomers with a substantial amount of acid and/or ester moieties. There is a carbohydrate domain in the sporopollenin and the relatively small amount of alkene/aromatic carbon shows that carotenoid monomers are not dominant in the polymer.

The dramatic decrease in the intensity of the δ10–55 region of the NMR spectrum when the dipolar dephasing condition is employed (Fig. 1) confirms the presence of CH_2 and CH_3 groups in the polymer [13]. The relatively small decrease in intensity of the bands at δ100–110, 130 and 150 ppm in the dipolar dephasing spectrum indicates that the carbons giving rise to these signals are substituted rather than protonated. Hence, the δ100–110 and 130 bands probably represent substituted alkene carbons such as one would find in carotenes. The resonance band centred at δ150 is characteristic of a quaternary aromatic carbon that is bonded to an electronegative group such as –OH or –OMe and may be due to the aromatic components proposed to be present in sporopollenin [6, 14].

The spectrum of exine material recovered from pollen which was treated with 4-methylmorpholine *N*-oxide at 20° was almost identical to that shown in Fig. 1 confirming the observation that treatment of pollen at 70° with 4-methylmorpholine *N*-oxide is a relatively mild isolation technique [11].

The ^{13}C NMR spectrum of lily sporopollenin (Fig. 1) is very similar to that of the plant polymer cutin which is

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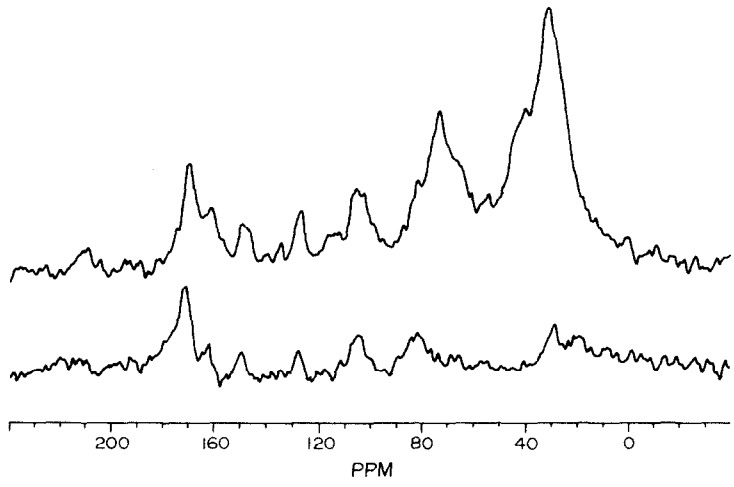

Fig. 1. Conventional cross polarization ^{13}C NMR (upper trace) and dipolar dephasing (lower trace) spectra of sporopollenin isolated from *Lilium longiflorum* pollen after treatment with 4-methylmorpholine *N*-oxide at 70°.

composed of hydroxy fatty acids esterified one to another to generate an insoluble polymer [15]. Cutin, however, is depolymerized by base hydrolysis while sporopollenin is resistant to this treatment [8]. Lily sporopollenin may be similar in chemical structure to the resistant, highly aliphatic polymer recently found in the cuticle of some plants [16, 17]. The spectrum in Fig. 1 is also very similar to that obtained from the aliphatic polymer isolated from the walls of the alga, *Botryococcus braunii* [18, 19], indicating that sporopollenin may possess a structure which is quite widespread. Aliphatic polymers which are highly resistant to degradation would be expected to play an important role in the formation of certain coals [20]. Although ultrastructural and chemical studies have shown that there is a wide diversity in sporopollenin form and composition [8, 21], the method of pollen exine isolation and characterization presented here would allow a detailed survey to determine if the type of aliphatic polymer found in lily pollen is characteristic of other sporopollenins.

EXPERIMENTAL

Lily pollen (*Lilium longiflorum* Thunb. cv Nellie White, Ace and Harbor) were harvested, stored at −20° and extracted with Me_2CO [11]. Pollen (500 mg) was suspended in a solution of 5 ml 1 M sucrose (which had been adjusted to pH 12.5 with 6 M NaOH) and 20 ml of aq. 60% 4-methylmorpholine *N*-oxide. The suspension was heated to 70° and stirred for 1 hr. A second prepn was suspended in the same manner, but the temp. held at 20° [22]. The exine fractions were recovered by a series (3) of differential centrifugations [2600 *g*, 15 min, 4°] through a discontinuous sucrose gradient (2 ml 0.70 M, 6 ml 0.76 M, 2 ml 1.78 M) [23]. The pelleted exine fractions were monitored by light microscopy to ensure complete removal of sporoplasts and washed with H_2O (× 3) and then Me_2CO to remove sucrose and 4-methylmorpholine *N*-oxide.

The 25.15 MHz cross polarization/magic angle spinning ^{13}C NMR measurements were obtained on a Bruker CXP-100 spectrometer as described previously [24].

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