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Preparation and Analysis of ^{14}C-Lignin Grass Lignocellulose and DHP

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ABSTRACT

(^{14}C-lignin) grass lignocellulose was prepared from wheat seedlings injected with (^{14}C-uniform) phenylalanine. Seedlings were injected 2 wk after germination and grown for 3–4 wk in a diurnal light cycle before harvesting. The plant material was ground in liquid N_2, extracted with hot water, benzene–ethanol, and ethanol, and treated with protease. Treatment of the lignocellulose with acid, alkali, and cellulase solubilized ^{14}C, which was analyzed by HPLC and TLC. Reverse-phase HPLC demonstrated that ^{14}C-ferulic and coumaric acid were bound primarily to carbohydrate and lignin, respectively. Gel permeation chromatography by HPLC of ^{14}C solubilized by treatment with 1*M* NaOH confirmed that the majority of the ^{14}C was incorporated into high molecular weight material. No ^{14}C was detected in either hexoses or pentoses obtained from the lignocellulose and only a minor amount was present as ^{14}C-phenylalanine. These studies show that (^{14}C-lignin) grass lignocelluloses must be carefully characterized before being used as defined substrates for biodegradation studies.

Coniferyl alcohol was synthesized by a route derived from those of Nakamura et al. (1974) and Nakamura and Higuchi (1976). DHP was then prepared by a modification of the method of Brunow and Wallin (1981) in which solutions of coniferyl alcohol and hydrogen peroxide were added alternately by a computer controlled HPLC system so that the coniferyl alcohol concentration was maintained below 1 m*M* throughout the synthesis. The DHP obtained was characterized by HPLC gel permeation chromatography and by NMR. The results of these analyses will be discussed.

REFERENCES

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