EVALUATION OF WOOD PRETREATMENTS ON OAK AND CEDAR

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ABSTRACT. In a separate study, we conducted a series of high-precision radiocarbon measurements using wood from Britain and New Zealand to investigate interhemispheric offsets and possible temporal variations. To minimize variability associated with different species, the pretreatment of the oak (*Quercus patraea*) and cedar (*Librocedrus bidwilli*) was to α-cellulose for both. This study investigates the thoroughness of a range of pretreatment processes by the stable isotope analysis of the products.

INTRODUCTION

To determine if observed differences between the radiocarbon dates of contemporaneous oak and cedar samples are representative of real atmospheric differences, it is essential that the chemical pretreatment of each follows the same pathway. An investigation into interhemispheric offsets between contemporaneous wood is being undertaken by The Queen's University of Belfast and the University of Waikato. A description of the project is outlined in McCormac *et al.* (1998). Previous calibration studies at Belfast on Irish oak (Pearson *et al.* 1986) have involved pretreatment to the holocellulose stage that is adequate for this species. However, when dealing with two species from different hemispheres, the material extracted for dating must be highly pure and consistently representative of the ^{14}C content of the atmosphere at the time of growth. To eliminate possible differences in the chemical composition of the material extracted for dating in the study, the pretreatment of both species is taken to a highly purified fraction, namely α-cellulose.

Several researchers, notably Wilson and Grinsted (1977), Tans and Mook (1980), and Leavitt and Long (1982) observed that the $\delta^{13}C$ of lignin is isotopically lighter than cellulose by up to 3‰. The presence of lignin in the pretreated end product can be detected reliably using $\delta^{13}C$ values (Sheu and Chiu 1994). Investigators, including Cain and Suess (1976), generally agree that all resin fractions should be discarded and that the amount of contamination by material from other years of growth depends on tree species.

Stuiver and Quay (1981) observed that on average, de Vries-pretreated wood (2% NaOH, 60°C, 2% HCl, 60°C) was 3.8 ± 1.4‰ higher in ^{14}C activity than α-cellulose from four sample pairs (post 1942) taken from a tree that grew from 1914–1975. Smaller differences were observed (0.7 ± 0.8‰) in ^{14}C activity between the de Vries-treated wood and α-cellulose of eight sample pairs, taken from the same tree pre-1939 (the heartwood-sapwood boundary). They assert that the de Vries method does not remove all material incorporated during the later stage of heartwood formation. They also observed the large increase in the ^{14}C activities of the de Vries wood after 1952 when nuclear bomb CO_2 was added to the atmosphere. They suggest that the pretreatment to α-celluose is particularly important if samples are taken from the later stage of the heartwood of a tree that survived into the nuclear era.

In this study, six pretreatment methods are used on two sets of contemporaneous oak and cedar samples. The $\delta^{13}C$ values of the pretreated samples were measured on a VG 602E mass spectrometer (McCormac *et al.* 1994). In all cases, three replicates were performed to give a measure of reproducibility.

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PROCEDURES

Section A: Pretreatments

A wood sample is planed or chipped prior to milling in a Thomas Wiley intermediate mill (size 20 mesh). The six pretreatment methods utilized in this study are outlined in a flow chart (Fig. 1).

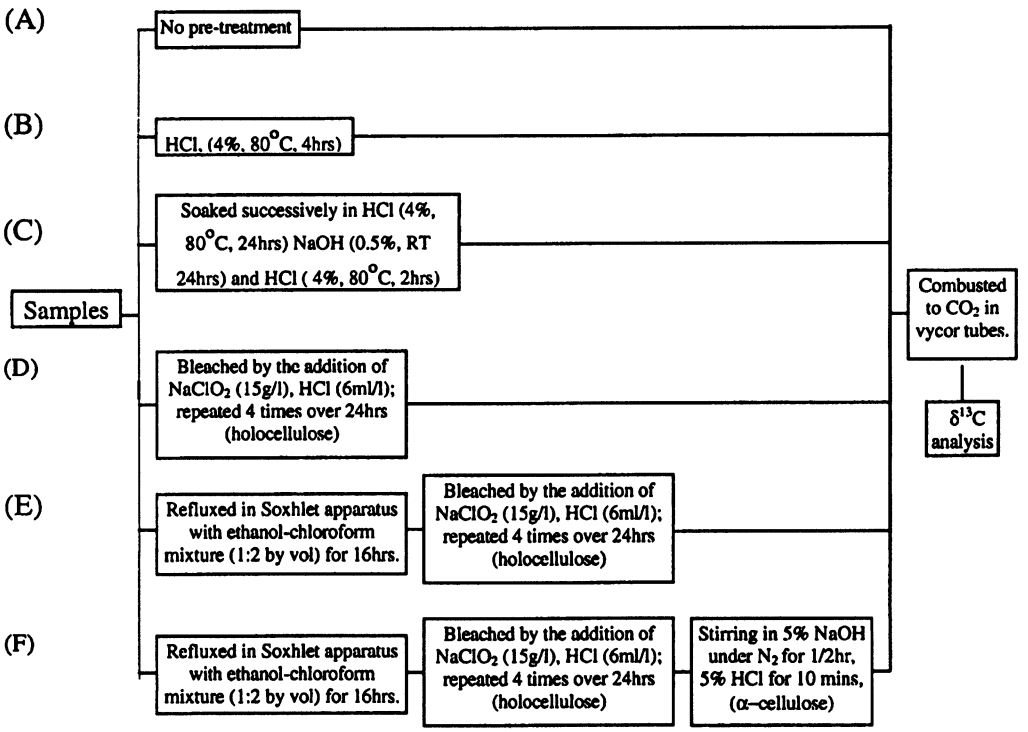

Fig. 1. Procedures evaluated in this study for extracting cellulose from wood samples for carbon stable isotope analysis. Letters in parentheses refer to the different methods (see text).

The methods differ in the use of organic solvents and the type of cellulose extracted. Method A involves no pretreatment and the raw wood is directly combusted to CO_2. Method B is a simple acid wash, whereas method C (AAA) follows that used by Tans and Mook (1980). Method D involves the extraction of holocellulose according to Green's method (1963). Method E (modified Green's method) is used to see the effect on lignin removal if the process is stopped at the holocellulose stage after extraction with organic solvents. Method F (α-cellulose) utilizes a three-step Soxhlet extraction process (ethanol/chloroform, ethanol and water). The organic solvents, which remove resins and other mobile contaminants, will be totally removed from the pretreated end product if this extraction sequence is followed. Subsequently, samples are converted to α-cellulose as shown in Figure 1. Method F (more detailed description in Fig. 2) is the method used for the extraction of cellulose from the interhemispheric calibration samples.

Section B: Stable Isotope Preparation and Analysis

The pretreated samples are combusted to CO_2 for $\delta^{13}C$ measurement using the technique as described by Boutton (1991). A 5–10-mg sample is placed in a clean Vycor® tube along with 400 mg

of purified copper oxide wire and a small piece of silver wool. This sample size range is chosen to avoid over-pressurizing the Vycor® combustion tubes while giving sufficient pressure in the inlet system of the mass spectrometer. The tube is evacuated and sealed. It is placed in a muffle furnace and the temperature is raised to 950°C for 1 h. After 1 h, the furnace is allowed to cool in stages (750°C for 1 hr and 550°C for 1 hr) before switching off. The sample tubes are cracked off-line and the gaseous products are cryogenically separated. The CO_2 samples are frozen down under liquid nitrogen into sample cylinders and removed for carbon stable isotope analysis.

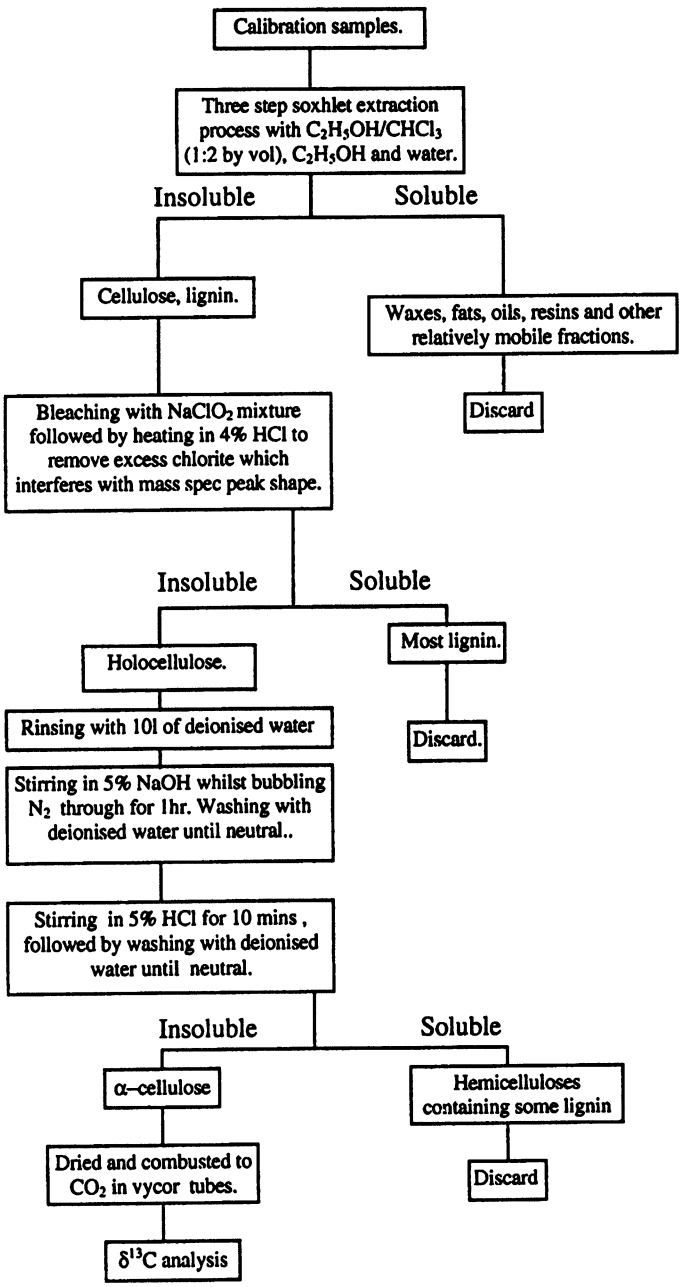

Fig. 2. The preferred method for the extraction of cellulose from interhemispheric calibration samples

RESULTS AND DISCUSSION

Isotope variation of natural abundances are quoted relative to PDB.

$$\delta^{13}C = \{[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{reference}]-1\} \times 1000‰ \quad (1)$$

To check the reproducibility of procedures and measurements, three replicates were carried out for each method evaluated. Two pairs of contemporaneous oak and cedar samples were analyzed. The results for the first pair are given in Table 1 (represented graphically in Fig. 3). It can be seen that there is up to a 1.2‰ depletion in $\delta^{13}C$ between whole wood and α-cellulose in oak, whereas in cedar the difference is 1.9‰. The results also show that the AAA method (C) is ineffective in removing lignin. In fact, the AAA pretreated wood is slightly more depleted than the whole wood for both species.

TABLE 1. Results of $\delta^{13}C$ analysis on two sets of contemporaneous oak and cedar pairs (decade centers 1605 AD and 1555 AD). Methods A–F refer to the pretreatment followed. Numbers are $\delta^{13}C$ (‰) values wrt PDB = mean of 3 replicates ± 2σ.

	First pair		Second pair	
Method	UB-5068 (oak)	UB-5069 (cedar)	UB-5078 (oak)	UB-5079 (cedar)
A	−25.84 ± 0.09	−22.58 ± 0.14	−25.91 ± 0.09	−23.24 ± 0.06
B	−26.25 ± 0.15	−22.84 ± 0.12	−26.20 ± 0.16	−23.43 ± 0.13
C	−26.32 ± 0.12	−22.90 ± 0.06	−26.47 ± 0.13	−23.47 ± 0.05
D	−24.74 ± 0.09	−21.11 ± 0.08	−24.83 ± 0.02	−21.99 ± 0.06
E	−24.70 ± 0.11	−21.14 ± 0.06	−24.75 ± 0.06	−22.03 ± 0.05
F	−24.68 ± 0.06	−20.69 ± 0.04	−24.83 ± 0.06	−21.39 ± 0.01

The study confirms that the holocellulose extraction technique as outlined by Green (1963) is adequate for the non-resinous oak, in that it removes all of the lignin. There is no change in the $\delta^{13}C$ in oak beyond holocellulose extraction. However, this was not found to be the case with cedar. This is seen in the $\delta^{13}C$ values, which are depleted by *ca.* 0.45‰ with respect to the α-cellulose. Hemicelluloses can contain up to 4% lignin and although Green's method would appear to remove all the lignin in oak, it does not in cedar. Cedar requires the use of alkali to remove the remaining lignin by dissolution of the hemicelluloses. Again from Table 1, we can see that findings were reproduced when the measurements were repeated on a second pair of contemporaneous oak and cedar samples.

For the second oak and cedar pair, the difference between whole wood and α-cellulose for oak is *ca.* 1.2‰, whereas that for cedar is 1.9‰. Both of these differences agree with the results from the first oak and cedar pair. Again, with cedar only, there is a clear difference of 0.6‰ between holocellulose and α-cellulose.

The α-cellulose measured for cedar sample UB-5079 was pretreated again using the bleach and alkali steps of procedure F to check for complete removal of $\delta^{13}C$-depleted lignin. Three samples of cellulose that had been processed once through procedure F and three that had been processed twice through F (excluding the organic soxhlet extraction) were measured again. The results showed no significant difference between the singly or doubly pretreated samples (single $\delta^{13}C$ = −21.32 ± 0.04‰, double pretreatment $\delta^{13}C$ = −21.33 ± 0.11‰). Thus, the degradation of the α-cellulose caused by further pretreatment did not alter the $\delta^{13}C$ value, confirming that a pure α-cellulose product is obtained by process F. This confirms that the chemical pretreatment is actually removing a depleted fraction (lignin) and not fractionating the end product by degradation of the cellulose.

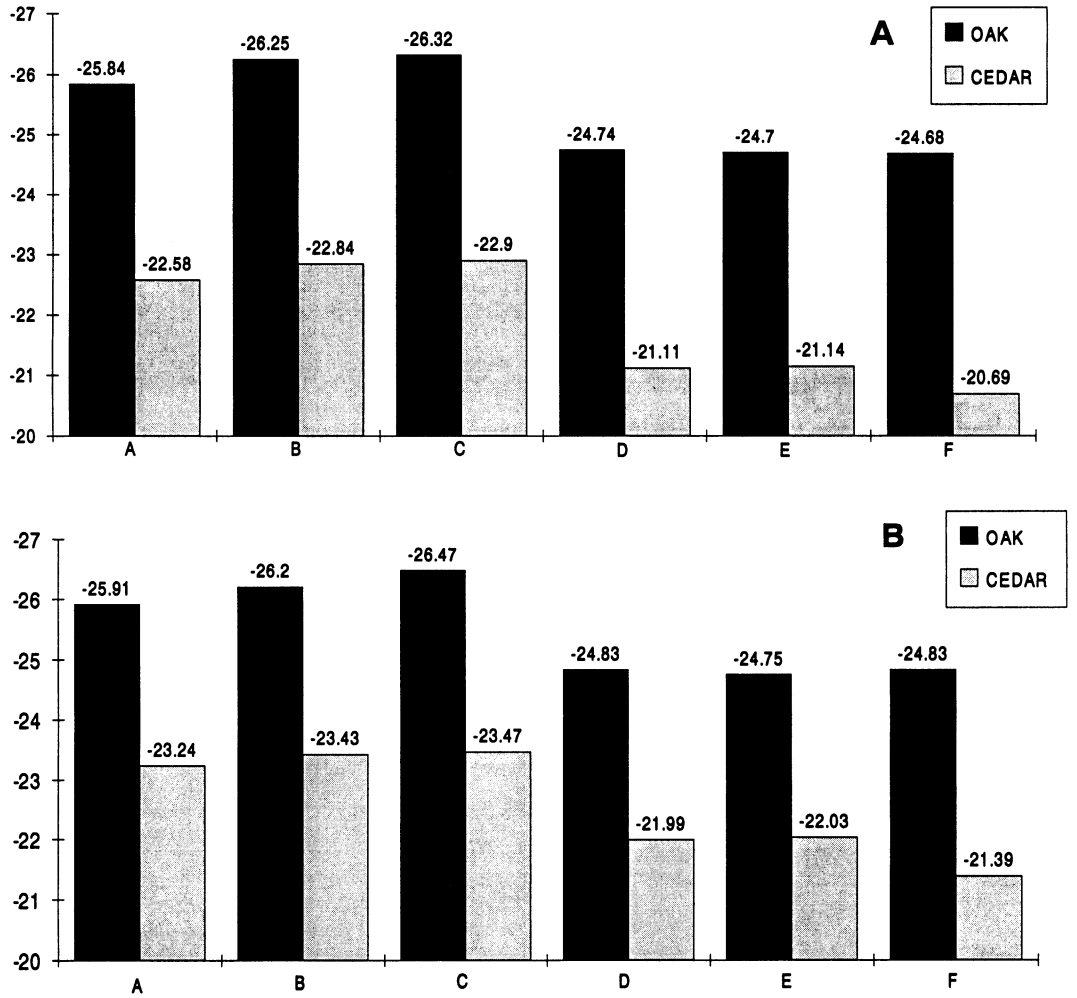

Fig. 3. Two bar graphs showing the $\delta^{13}C$ values of oak and cedar sample pairs pretreated using methods A–F; A=sample pair centered on 1605 AD; B=sample pair centered on 1555 AD.

Conclusion

This carbon stable isotope study has shown that lignin can remain unless cedar is pretreated to α-cellulose. The two species, oak and cedar, differ in that oak seems to be lignin free at the holocellulose stage, whereas cedar requires further treatment to α-cellulose to remove all of the lignin. The enrichment that is observed on going from whole wood to α-cellulose is due to the removal of $\delta^{13}C$-depleted lignin and not due to fractionation in $\delta^{13}C$ of the cellulose caused by the pretreatment itself. Each species must be treated in exactly the same way to validate any interhemispheric offsets that are found. By following method F (α-cellulose), for both species, we are ensuring that the fraction isolated for dating is as representative as possible of prevailing atmospheric ^{14}C concentration at the time of growth.

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