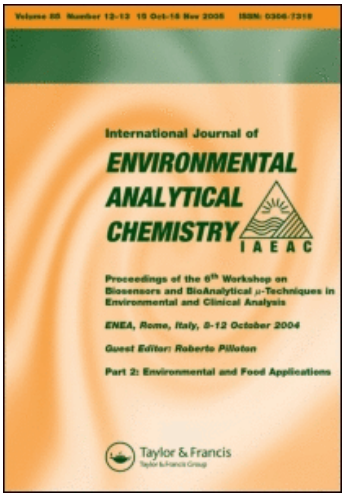

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EVALUATION OF CELLULOSE EXTRACTION PROCEDURES FOR STABLE CARBON ISOTOPE MEASUREMENT IN TREE RING RESEARCH

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Evaluation of cellulose extraction procedures for preparing tree ring samples for carbon isotopic analysis reveals that cellulose can be best extracted with a C_6H_6-C_2H_5OH mixture in a Soxhlet apparatus and then soaked and bleached in $NaClO_2$ + CH_3COOH solutions. Moreover, systematic measurements of $\delta^{13}C$ of tree rings show that they vary considerably across rings and also vary around rings in different radial directions, a fact of great importance in interpretation of tree ring $\delta^{13}C$ time series records for past climatic conditions.

KEY WORDS: Cellulose extraction, tree ring, carbon isotope.

INTRODUCTION

Recent concerns on the increase of atmospheric CO_2 and its associated global warming phenomenon have challenged scientists to seek and document various natural archives that retain records of the past climatic conditions. Among these, studying the tree ring has been shown to be the one with great potential because of its close response to atmospheric CO_2 changes and the uninterrupted, high (annual to seasonal) resolution character.[1] For instance, the continuing decrease in carbon isotope compositions ($\delta^{13}C$) of tree rings has been attributed to the addition of ^{13}C-depleted CO_2 in the atmosphere from fossil fuel combustion and land deforestation.[2-7] As a consequence, analysis of tree ring $\delta^{13}C$ and compilation of these data in a global scale have provided important clues to a better understanding of the past climate changes. Nonetheless, methods of extracting cellulose from tree rings for carbon isotopic measurements reported in the literature are inconsistent and thus make use of results from these studies for synthesizing the global tree ring $\delta^{13}C$ database inconclusive. This inconsistency may also account for the controversy on anticipated decrease in tree ring $\delta^{13}C$ due to the release of ^{13}C-depleted CO_2 from fossil fuel combustion and deforestation. It is, therefore, the purpose of this study to evaluate systematically various cellulose extraction procedures fro carbon isotope analysis. Additionally, we also reported results from measurements of $\delta^{13}C$ across several consecutive annular rings and around each ring in different radii to show their seasonal and circumferential variations, a subject that needs caution in interpreting tree ring data for climatic reconstruction.

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MATERIALS AND CELLULOSE EXTRACTION

Tree ring samples

Tree ring samples analyzed in the study were drilled with a standard increment borer (20″ x 0.2″) from one stand of fir *(Abies Kawakamii (Hayata) Ito)* growing in an open ground at an elevation of approximately 3,844 meters in southern Taiwan. Additional samples were obtained from a cross section cut off from trunk of hemlock from southwest China. Upon return to the laboratory, individual tree ring sample was chipped off using a surgery scalpel and stored in a glass vial. Furthermore, samples used for studying seasonal variations in $\delta^{13}C$ were separated into four equal subdivisions across an annular growth ring. All samples thus collected were then subjected to extraction of cellulose and analysis of carbon isotopic compositions.

Cellulose extraction procedures

Figure 1 shows different cellulose extraction procedures tested in this study for preparing tree ring samples for stable carbon isotope measurements. As can be seen, the methods differ mainly in the use of different solvents and whether samples were soaked in flask or refluxed in a Soxhlet apparatus. Method 1 is by directly combusting raw wood samples to CO_2 without any pretreatment and results are used to compare with those obtained from other methods. Method 2 is mainly the digestion of samples in HCl-NaOH-HCl solution and is following that of Tan and Mook[8]. In method 3 tree ring samples are soaked in an ethanol-benzene (C_6H_6-C_2H_5OH) mixture, while samples are refluxed with the same solvent in method 4. Method 5 includes an additional step for α-cellulose extraction as compared to method 4. After a thorough investigation (see discussion below), method 4 was used as a standard method for extracting cellulose from tree ring samples throughout this study and shown in Figure 2.

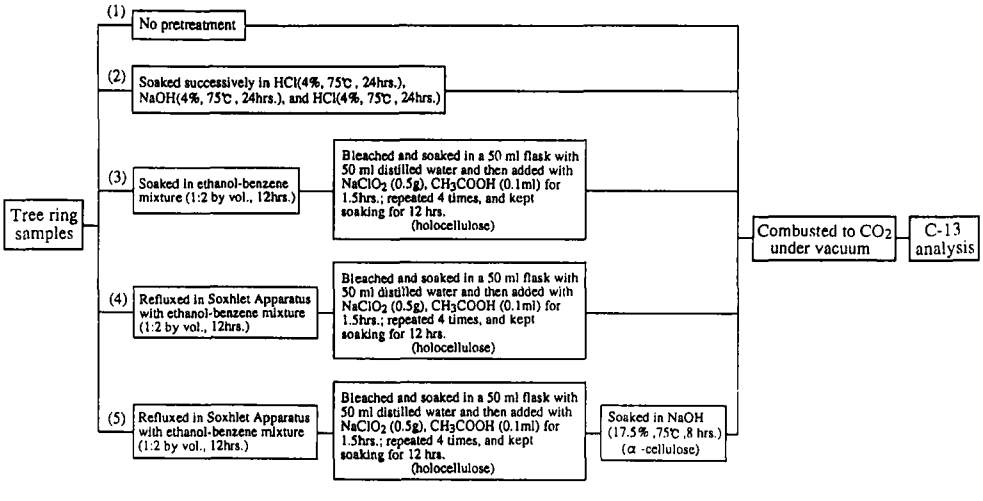

Figure 1 Various procedures evaluated in this study for extracting cellulose from tree rings for carbon isotopic analysis. Numbers in parentheses refer to the different methods (see text).

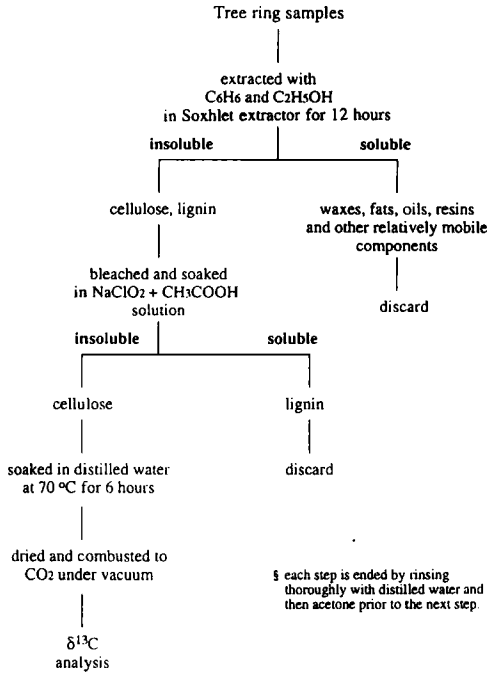

Figure 2 A flow chart showing method of cellulose extraction recommended in this study for preparing tree ring samples for carbon isotopic measurements.

Carbon isotopic analysis

Preparation of raw wood and cellulose samples for carbon isotopic analysis followed the routine procedures of Craig[9]. Samples were combusted to CO_2 gas at 850°C in a standard glass preparation line under vacuum. Water was removed by a slurry of dry ice and alcohol mixture, while CO_2 was condensed, purified and collected using a liquid N_2 trap. The resulting CO_2 was then analyzed for $\delta^{13}C$ using a VG Optima isotopic ratio mass spectrometer (IRMS). Results were reported in the conventional δ notation relative to the PDB standard, where

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

The analytical precision of IRMS estimated from our laboratory standards was better than ± 0.05 ‰. In this study all samples were analyzed in replicates except for a few samples that were analyzed only once due to the small amount of samples available.

RESULTS AND DISCUSSION

Evaluation of cellulose extraction methods

It has been well known that wood is composed mainly of cellulose, lignin and other minor components, and their carbon isotopic compositions are different and variable

Table 1 Results of $\delta^{13}C$ measurements on tree ring samples extracted by various methods (except for method 1, directly combusting the raw woods without pretreatment is used for comparison with other methods). Values in parentheses are those measured in duplicates only on cellulose.

Method	*(1)*	*(2)*	*$\delta^{13}C$ (‰)* *(3)*	*(4)*	*(5)*
Replicates					
1	-24.83	-24.91	-23.60	-23.41	-23.15
		(-23.76)	(-24.39)	(-23.97)	(-24.16)
2	-25.08	-24.82	-23.12	-23.27	-23.03
		(-24.44)	(-24.46)	(-23.38)	(-23.83)
3	-25.90	-25.11	-23.53	-23.46	-23.11
		(-25.53)	(-24.54)	(-24.00)	(-24.12)
4	-25.36	-25.43	-23.49	-23.30	-23.22
		(-26.75)	(-24.96)	(-24.76)	(-24.60)
Average	-25.29±0.4	-25.09±0.88	-24.01±0.65	-23.69±0.51	-23.65±0.60

among trees. As a consequence, isotopic measurement of tree rings is usually performed on cellulose because of its chemical stability and physiological sensitivity to environmental changes as compared to other components.[10–13]

Table 1 shows results of $\delta^{13}C$ measurements of tree ring samples extracted with different methods. As shown, values of $\delta^{13}C$ in raw woods are invariably lighter than those being extracted for cellulose. Since lignin is known to be isotopically lighter than cellulose up to approximately 3 o/oo[12], the observed lighter isotopic ratios would indicate the presence of C-13 depleted lignin in the samples after being extracted. The results are consistent with previous observations.[8,10,13,14,15] Nonetheless, data also show that isotopic measurement of raw wood is characteristic of a relatively smaller spread in replicates than those of being extracted for cellulose.

In addition, values of $\delta^{13}C$ in cellulose extracted by a mixture of HCl-NaOH-HCl solution (method 1) are 1 – 1.5 o/oo lighter tan those extracted by organic solvents. Because the method has been widely used in the literature to extract cellulose for isotopic measurements, results from our experiments show that caution needs to be taken for data comparison and that a systematic correction must be applied for compilation and synthesis of global tree ring $\delta^{13}C$ database.

Finally, refluxing of tree ring samples seems to result in a relatively heavier $\delta^{13}C$ than soaking with the same organic agents. Accordingly, a typical trend of $\delta^{13}C$ measurements using various techniques can be established as follows: $\delta^{13}C$ (refluxed with ethanol-benzene) > $\delta^{13}C$ (soaked with ethanol-benzene) > $\delta^{13}C$ (soaked with HCl-NaOH-HCl) > $\delta^{13}C$ (raw wood). We also observed that the cellulose extracted with method 4 consistently gave extracted cellulose of a whiter appearance. Although judgment on selection of methods suitable for cellulose extraction cannot be made solely on the basis of isotopic results, measurements of $^{13}C/^{12}C$ ratios in cellulose extracted by method 4 seem to give a better reproducibility. As a consequence, method 4 is recommended to be the standard method for extraction of cellulose from tree ring samples for carbon isotopic analyses and was used throughout this study.

Table 2 $\delta^{13}C$ values of equal subdivisions of annular rings of fir from southern Taiwan (A) and hemlock from southwest China (B). Values in parentheses are those measured in duplicates.

A. fir, Taiwan

	$\delta^{13}C$ (‰)				
Ring No.	*1*	*2*	*3*	*4*	*Average§*
1953	-23.83	-23.32	-23.10	-23.70	-23.48
1954	-23.40	-23.59	-23.02	-23.20	-23.30
1955	-23.71	-24.06	-22.71	-23.20	-23.42
1956	-24.30	-22.97	-23.54	-23.25	-23.51
1957	-23.23	-23.80	-23.21	-22.47	-23.17

B. hemlock, southwest China

	$\delta^{13}C$ (‰)					
Ring No.	*1*	*2*	*3*	*4*	*Average§*	*Bulk**
1	-22.65 (-21.91)	-23.03 (-23.01)	-22.56 (-22.56)	-22.12 (-22.50)	-22.54±0.34	-22.55±0.04
2	-22.05 (-21.43)	-23.59 (-23.07)	-23.19 (-23.21)	-23.69 (-23.93)	-23.02±0.89	-22.41±0.06
3	-22.33 (-22.22)	-23.11 (-23.85)	-22.97 (-22.34)	-22.73 (-22.51)	-22.59±0.02	-22.65±0.19
4	-21.82 (-21.75)	-22.70 (-22.69)	-22.26 (-22.80)	-23.60 (-23.60)	-22.65±0.50	-22.89±0.07

§ averaged $\delta^{13}C$ values of 4 subdivisions.
* $\delta^{13}C$ values measured from one single ring.

Seasonal variations across a single ring

It is known that a tree grows only within a certain range of temperature in a year and different trees have their own specific growing seasons. The growth results in circular layers (annular rings) as viewed from a cross section. Furthermore, climate, e. g. temperature and precipitation, also affects growth of the rings. In general, early wood that grows during the spring season is characteristic of less dense and light color, while late wood that grows in late summer and early fall is dense and dark in color. It has also been commonly observed that $\delta^{13}C$ of the early wood is isotopically lighter than the late wood regardless of the tree species.[1,5,10,13,15]

Table 2 lists the seasonal variations in $\delta^{13}C$ of tree rings of fir from Taiwan and hemlock from China, respectively. The variation is further depicted in Figure 3. As can be seen, they vary between -22.47 and -24.30‰ across five consecutive rings of fir and between -21.43 and -23.93‰ across four consecutive rings of of hemlock. Furthermore, the variations across a single annual ring appear to differ between rings. We have also analyzed the bulk isotopic compositions of individual rings of hemlock (Table 2). According to the Table, the difference between the averaged $\delta^{13}C$ and bulk $\delta^{13}C$ of each individual ring is variable and ranges from 0.01 to 0.61‰ in our experiments, thus making the feasibility of using bulk $\delta^{13}C$ of tree rings in constructing their time series variations for past climatic study somewhat ambiguous.

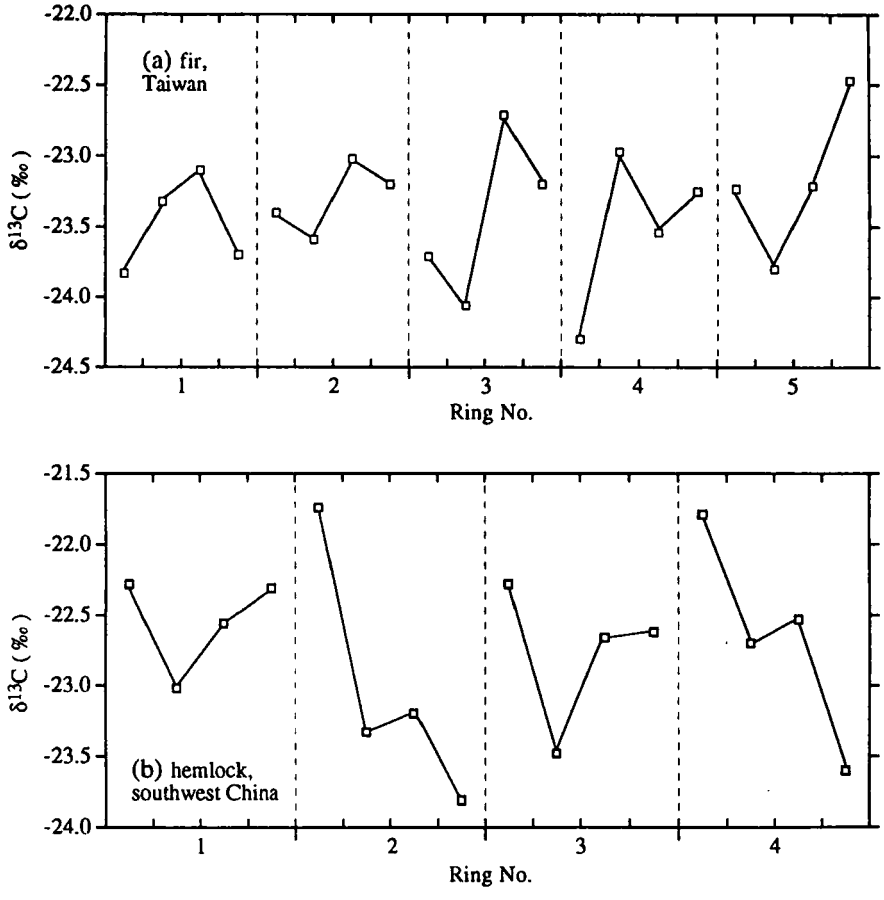

Figure 3 Variations in $\delta^{13}C$ across several consecutive rings of fir from southern Taiwan (a) and of hemlock from southwest China (b).

Circumferential variation around a single ring

Table 3 lists values of $\delta^{13}C$ measured in different radial directions of fir from 1950 to 1992, while Figure 4 depicts ranges of their variations in $\delta^{13}C$ and the trend of the averaged $\delta^{13}C$. As can be seen, with a few exceptions they covary fairly well and are well within 1‰ deviation of $\delta^{13}C$. It has been reported that $\delta^{13}C$ around an individual ring could vary up to 2‰ among radial directions.[8,16,17] The results of isotopic analysis of fir from Taiwan in this study are thus consistent with previous observations.

CONCLUSIONS

Studying high resolution tree ring records has been identified as one of the major research areas in the Past Global Changes (PAGES) project of the International Geosphere-Biosphere Programme (IGBP). Among others, compilation and synthesis of

Table 3 List of $\delta^{13}C$ in three different directions of Taiwan fir from 1950 to 1992 and their average values.

	$\delta^{13}C$ (‰)			
YEAR AD	*A (NORTH)*	*B (WEST)*	*C (EAST)*	*AVERAGE*
1950	-23.06±0.04	-23.76±0.09	-22.61±0.14	-23.14
1951	-23.07±0.00	-23.49±0.02	-22.65±0.07	-23.07
1952	-23.56±0.23	-23.40±0.03	-22.63±0.09	-23.20
1953	-23.55±0.04	-23.62±0.14	-23.13±0.01	-23.43
1954	-23.26±0.16	-23.42±0.07	-23.33±0.25	-23.34
1955	-23.36±0.10	-23.44±0.01	-23.59±0.20	-23.46
1956	-23.62±0.16	-23.15±0.13	-23.40±0.07	-23.39
1957	-23.22±0.11	-23.60±0.19	-23.18±0.09	-23.33
1958	-23.60±0.01	-23.34±0.20	-23.19±0.08	-23.38
1959	-23.52±0.02	-23.19±0.11	-24.15±0.34	-23.62
1960	-23.35±0.12	-23.41±0.07	-23.39±0.05	-23.38
1961	-23.63±0.11	-23.24±0.01	-23.51±0.17	-23.46
1962	-23.44±0.06	-23.34±0.13	-23.51±0.08	-23.43
1963	-23.46±0.19	-22.89±0.01	-22.92±0.14	-23.09
1964	-23.58±0.02	-23.42±0.12	-22.87±0.00	-23.29
1965	-23.51±0.28	-22.87±0.02	-22.60±0.04	-22.99
1966	-23.33±0.20	-23.33±0.04	-23.05±0.34	-23.24
1967	-23.52±0.04	-23.11±0.03	-22.87±0.04	-23.17
1968	-23.29±0.16	-22.70±0.11	-22.67±0.06	-22.89
1969	-23.35±0.19	-22.72±0.06	-22.51±0.17	-22.86
1970	-23.25±0.12	-22.88±0.18	-22.65±0.31	-22.93
1971	-23.71±0.27	-23.29±0.19	-23.25±0.16	-23.42
1972	-23.02±0.02	-22.83±0.01	-22.88±0.07	-22.91
1973	-23.57±0.08	-23.57±0.12	-23.24±0.36	-23.46
1974	-23.45±0.24	-22.98±0.02	-22.15±0.27	-22.86
1975	-23.67±0.08	-23.53±0.26	-22.76±0.31	-23.32
1976	-23.30±0.12	-22.90±0.14	-22.75±0.04	-22.98
1977	-23.29±0.06	-23.07±0.13	-22.94±0.26	-23.10
1978	-23.15±0.07	-23.11±0.11	-23.30±0.04	-23.19
1979	-23.75±0.01	-23.02±0.06	-23.19±0.13	-23.32
1980	-23.77±0.05	-23.54±0.01	-24.31±0.38	-23.87
1981	-23.35±0.05	-23.79±0.00	-23.54±0.22	-23.56
1982	-23.54±0.01	-23.57±0.11	-23.39±0.12	-23.50
1983	-23.75±0.07	-23.57±0.06	-23.54±0.18	-23.62
1984	-23.73±0.14	-23.08±0.34	-23.60±0.17	-23.47
1985	-23.13±0.08	-23.33±0.22	-23.74±0.08	-23.40
1986	-23.57±0.20	-24.07±0.17	-23.81±0.23	-23.82
1987	-23.49±0.15	-23.08±0.04	-23.75±0.27	-23.44
1988	-24.24±0.01	-24.21±0.32	-23.80±0.18	-24.08
1989	-24.28±0.13	-23.83±0.09	-23.47±0.08	-23.86
1990	-23.39±0.26	-23.80±0.37	-23.42±0.42	-23.54
1991	-23.40±0.04	-23.47±0.06	-23.07±0.09	-23.31
1992	-23.36±0.16	-23.52±0.02	-23.55±0.02	-23.48

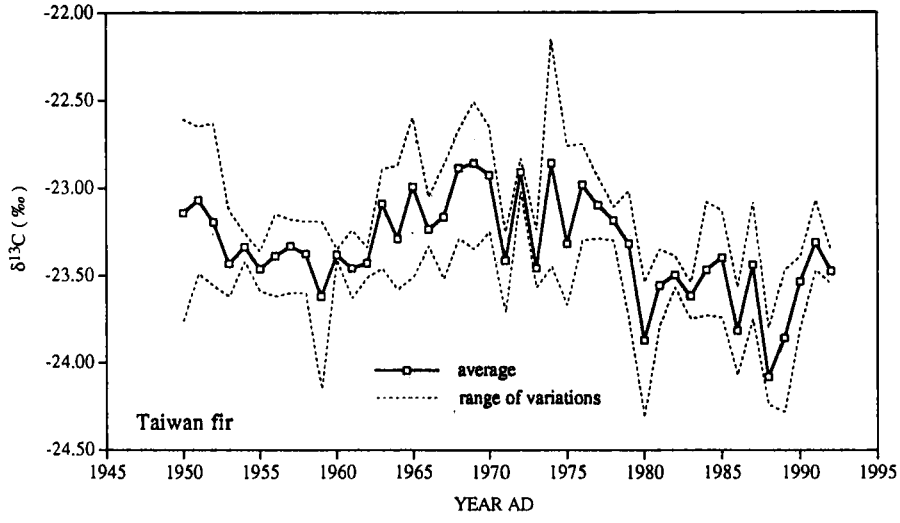

Figure 4 Range of $\delta^{13}C$ variations from 1950 to 1992 around rings in different radial directions (dashed line) and the time series variations of averaged $\delta^{13}C$ (solid line) of fir from southern Taiwan.

tree ring data on a global scale are essential to the success of the project. However, since methods for extracting cellulose for carbon isotopic analysis have been controversial in the literature, there is an urgent need to evaluate these procedures for comparison of results from various laboratories. Furthermore, while $\delta^{13}C$ variations found across an annular growth ring may imply for seasonal changes in climatic conditions, the variations observed around rings in different radii may more likely be attributed to the physiology of the tree. Results from this study may, therefore, shed light on the use of tree ring $\delta^{13}C$ records for interpreting past climatic conditions.

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