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On the purification of α-cellulose from resinous wood for stable isotope (H, C and O) analysis

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Abstract

α-Cellulose was isolated from four samples of Scots pine (*Pinus sylvestris* L.). Each sample was divided into two portions. One portion had the resins removed by solvent extraction prior to removal of lignins by treatment with acidic sodium chlorite solution and treatment with sodium hydroxide solution to remove hemicelluloses. The other portion was processed in the same way apart from the solvent extraction step. The isolated wood constituents were characterised by attenuated total reflectance Fourier transform infrared (ATR/FT-IR) spectroscopy. The infrared spectra of the resulting α-cellulose samples were identical indicating that treatment with acidic sodium chlorite and sodium hydroxide was sufficient to remove resins. The values of the stable isotope ratios (carbon, oxygen and hydrogen) for each pair of α-cellulose sub-samples also showed no significant differences within the reproducibility of the methods. The implication of these studies demonstrate that the customary step of resin extraction from pine is unnecessary if sodium chlorite and sodium hydroxide are used for the isolation of α-cellulose following the technique described in this paper. In addition, the study demonstrates that the oxygen isotope ratio of the water used for cellulose extraction does not influence the stable isotope values in the α-cellulose obtained. The importance of isotopic homogeneity within the cellulose sample is also highlighted.

Keywords: Stable isotopes; Pine; Resin; Solvent extraction; Infrared spectroscopy; Cellulose; Linked water

1. Introduction

Ratios of stable isotopes of carbon, oxygen and hydrogen (measured as $\delta^{13}C$, $\delta^{18}O$ and $\delta^{2}H$ respectively) in the annual growth rings of trees are valuable sources for the reconstruction of past climates and for ecophysiological research (McCarroll and Loader, 2004; Switsur and Waterhouse, 1998). The physical and physiological factors causing isotopic discrimination when trees use CO_2 and H_2O as the starting materials for the synthesis of trunk cellulose

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are relatively well known for carbon (Farquhar et al., 1982; Saurer et al., 1997; Berninger et al., 2000) and for oxygen and hydrogen (Roden et al., 2000; Anderson et al., 2002; Waterhouse et al., 2002). This has enabled the use of stable isotopes in tree rings as climatic proxies in regions where ring width analysis shows little correlation to climatic parameters. In most of the cases models have been able to produce high correlations between isotope values and climate parameters (e.g. temperature, precipitation and humidity) from various environments back throughout the Holocene (Robertson et al., 1997; McCarroll and Pawellek, 2001; Sheu et al., 1996; Becker et al., 1991).

Stable isotopes in tree rings hence offer a very promising method for reconstructing precisely dated annual climatic history not only from living but also from subfossil trees (Mayr et al., 2003). Indeed, by carefully sampling wood from across annual growth rings, it may be possible to extract climatic information at much higher temporal resolution (Loader et al., 1995; Barbour et al., 2002). A recent study, however, has revealed the complexity of the factors involved in determining behaviour of the $\delta^{13}C$ signal at high resolution through an annual growth ring (Helle and Schleser, 2004). Exploitation of this record has so far been restricted owing to very labour intensive and time consuming preparation of many thousands of samples for isotope measurements. Improvements that can shorten these processes would be of great benefit, increasing both the length of individual series that can be obtained and the ability to replicate time series.

A variety of methods is available for the preparation of α-cellulose or holocellulose from wholewood for isotopic analysis (Green, 1963; Leavitt and Danzer, 1993; Loader et al., 1997; Brendel et al., 2000). The use of cellulose as a sample material, as opposed to wholewood or lignin, has been standard practice since Wilson and Grinsted (1977) showed that different wood components have different isotopic ratios. Variation in the content of chemical components across annual rings could potentially weaken the climatic signal obtained if wholewood only were to be used. The mobility of resin (Long et al., 1979) and the later deposition time for lignin compared to cellulose (Wilson and Grinsted, 1977) have also been factors against the use of wholewood. These arguments remain valid particularly in studies attempting to model complex plant–environment systems. Recent findings suggest that for the study of palaeoclimate, where the aim is to maximise precision of the estimate of the mean, rather than the precision of individual measurements (McCarroll and Loader, 2004), the climatic signal in cellulose and (resin extracted) wholewood may provide broadly equivalent information (Barbour et al., 2001; Loader et al., 2003). However, they also identify that where trees may have suffered diagenesis such records should be interpreted with care.

When conifer wood is used, it is normal first to remove resins and other soluble materials by solvent extraction before other chemical treatments. Samples are treated using a suitable solvent, e.g. 2:1 toluene–ethanol, for up to 8 h, washed free of solvent and dried at around 40° in a vacuum oven overnight. In this study we investigate the necessity of the solvent extraction stage. To do this we measured stable isotopic ratios ($\delta^{13}C$, $\delta^{18}O$ and $\delta^{2}H$) of α-cellulose extracted from samples of Scots pine, with and without a solvent extraction stage. Scots pine was chosen because this highly resinous species is used in the ISONET-project (400 years of annual reconstructions of European climate variability using a high resolution isotopic network) of which this study forms a small part. We used ATR/FT-IR to follow the composition of material at the various stages from whole wood to α-cellulose. Infrared spectroscopy has been used since the 1950s (Tschamler et al., 1953) in the analysis of wood, and has become a very effective method in analysing the chemistry of wood constituents and the changes that occur in wood due to different chemical treatments (Pandey, 1999; Moore and Owen, 2001).

It is known that cellulose is extremely hydrophilic. Scheirs et al. (2001) report that total removal of water linked to cellulose-OH requires temperatures up to 220 °C, and even then water will rapidly reappear after heating. Indeed, during this study, it became apparent from IR spectra that residual water was still present in cellulose, even after prolonged drying in a vacuum oven (see below). We therefore extended the study to see whether water used during the extraction process could influence the $\delta^{18}O$ of the cellulose. This was done by carrying out the extraction in water

enriched in ^{18}O and checking for possible enhancement of the $\delta^{18}O$ value of the resulting cellulose.

2. Materials and methods

Four samples of wood from Scots pine (*Pinus sylvestris* L.) were used for this study. A tree from Northern Finland provided one sample of wood. Another tree was from the UK, and this provided two samples: one from the heartwood and one from the sapwood. The fourth sample was a mixture wood from five well preserved trees lying on the surface in the mountain tundra above the modern tree-line in the Kola Peninsula, Russia; these sub-fossil pines had radiocarbon ages between 910 and 650 ^{14}C years BP (Hiller et al., 2001). Samples 2 and 4 were obtained from heartwood only. The material from UK and Finland was initially cut into shavings and then homogenized into small particles using a coffee grinder. The very dry wood from the Kola sub-fossil trees had been homogenized by grounding to a powder and sieving (<400 μm mesh). Each of the four samples was then divided into two fractions: from one half α-cellulose was prepared with the solvent extraction stage, from the other α-cellulose was prepared without solvent extraction. With this exception samples were treated identically and α-cellulose prepared following the method of Loader et al. (1997).

Solvent extraction was carried out in a Soxhlet apparatus using a 2:1 mixture of toluene/ethanol. Lignins were oxidized using an acidified sodium chlorite solution and ultrasonic bath at 70 °C until the product, holocellulose, was white. This could take up to seven or eight separate additions. The samples were then treated in an ultrasonic bath in 10% sodium hydroxide solution for 45 min at 80 °C, and then in 17% sodium hydroxide solution for 45 min in room temperature in an ultrasonic bath to leach hemicelluloses, such as mannan and xylan (Loader et al., 1997). α-Cellulose samples were dried in a vacuum oven at 40 °C over night. After each step in the chemical treatment a subsample was taken from all the eight treated wood samples for further analysis.

IR spectra were measured using a Perkin Elmer Spectrum One FT IR Spectrometer equipped with a Perkin Elmer Universal ATR Sampling Accessory. Each spectrum was measured at a spectral resolution of 4 cm^{-1} and 5 scans were taken per sample.

Values of $\delta^{13}C$ were determined using dry α-cellulose (200–300 μg), which was weighed into individual tin foil cups and combusted "on-line" at 1000 °C using an ANCA GSL elemental analyzer interfaced to a PDZ Europa 20/20 isotope ratio mass spectrometer. To measure $\delta^{18}O$ values, the dry α-cellulose samples (ca. 200 μg) were weighed in silver capsules, pyrolysed at 1450 °C and measured "on-line" with a HT-O, HEKAtech high temperature pyrolysis reactor coupled with a ThermoFinnigan DELTA plus XL isotope ratio mass spectrometer. Measurements of $\delta^{2}H$ of non-exchangeable carbon-bound hydrogen were carried out on the cellulose nitrate of the wood samples. The cellulose was nitrated at room temperature using the modified methods of Alexander and Mitchell (1949) and of Gray and Song (1984). Cellulose nitrate samples (ca. 400 μg) were weighed in silver capsules, pyrolysed "on-line" at 1450 °C and hydrogen produced passed into a ThermoFinnigan IRMS-DELTA plus XL isotope ratio mass spectrometer (Knöller et al., in preparation). Results are reported using conventional notation $(\delta = (R_{sample} - R_{standard})/R_{standard} \times 1000‰$; where $R = {}^{13}C/{}^{12}C$ (with respect to the V-PDB standard), $^{18}O/^{16}O$ or D/H (with respect to the VSMOW standard). The overall precision of the replicate samples analyses is estimated to be better than 0.1‰ for $\delta^{13}C$, 0.3‰ for $\delta^{18}O$, and 2‰ for $\delta^{2}H$.

A sample of oak composed of wood from several years of growth was used to check whether waters used in the extraction procedure measurably altered the isotopic composition of the final isolated cellulose. One litre of water enriched in ^{18}O ($\delta^{18}O = 29.6‰$) was prepared by dilution of water containing 95 at.% ^{18}O (ex Aldrich). The study could equally well have been carried out using ^{2}H-enriched water, but this would have lengthened the process due to nitration of cellulose. All solutions of acetic acid, sodium chlorite and sodium hydroxide were prepared using this solution. Samples of α-cellulose were dried in a vacuum oven for 3 days at 70 °C.

For comparison of with the resulting IR spectra a standard sample of α-cellulose powder, 'Fluka α-cellulose', was manufactured from spruce by Fluka Chemie GmbH, Switzerland and supplied by Sigma-Aldrich GmbH, Taufkirchen, Germany.

3. Results and discussion

3.1. Comparison of resin extracted and non-resin extracted cellulose

The effect of each chemical treatment on the IR absorption bands when using the conventional toluene–ethanol solvent extraction is shown in Fig. 1. Infrared spectra of wood samples are complex and contain many bands that are broad and often overlap with neighbouring bands. Especially below 1460 cm^{-1} they have contributions from various vibration modes in resin, lignin and carbohydrates. The most characteristic changes in the spectra during the extraction of α-cellulose occur on the wavelengths marked in Fig. 1. The effect of solvent extraction can be clearly seen as a disappearance of the peak 2 (1690 cm^{-1}) arising from C=O bonds (Browning, 1967). The spectra show that resin has quite strong absorption also between peaks 1 (1732 cm^{-1}) and 4 (1604 cm^{-1}). Of the pine samples chosen for this study, the sample represented in Fig. 1 (heartwood) had the greatest amount of resin as indicated by the height of the resin-derived absorbance. Previous studies on Scots pine have reported functional groups of lignin absorbing around positions of the peaks 1, 3 (1649 cm^{-1}), 4 and 5 (1500 cm^{-1}) (Pandey and Pitman, 2003). The latter peak seems to have the most prominent decrease after the acidified sodium chloride treatment. The sodium hydroxide stage removes peak 1, which arises from hemicellulose (Pandey and Pitman, 2003). The treatment would also seem to remove residues of lignin, which is indicated by the disappearance of the lignin-derived peaks 4 and 5. Peak 3 represents the only peak in the region between 1740 and 1510 cm^{-1} that still absorbs after the completion of the chemical treatments. Based on previous studies it seems to be caused solely by water linked to cellulose-OH (Harrington et al., 1964). A more detailed description of the infrared bands of Scots pine is presented by Pandey and Pitman (2003).

Fig. 2 shows the IR absorption bands for the same sample in the different stages of the chemical procedure when solvent extraction is not used. The result implies that the resin was removed from the whole wood during the treatment with acidified $NaClO_2$ and

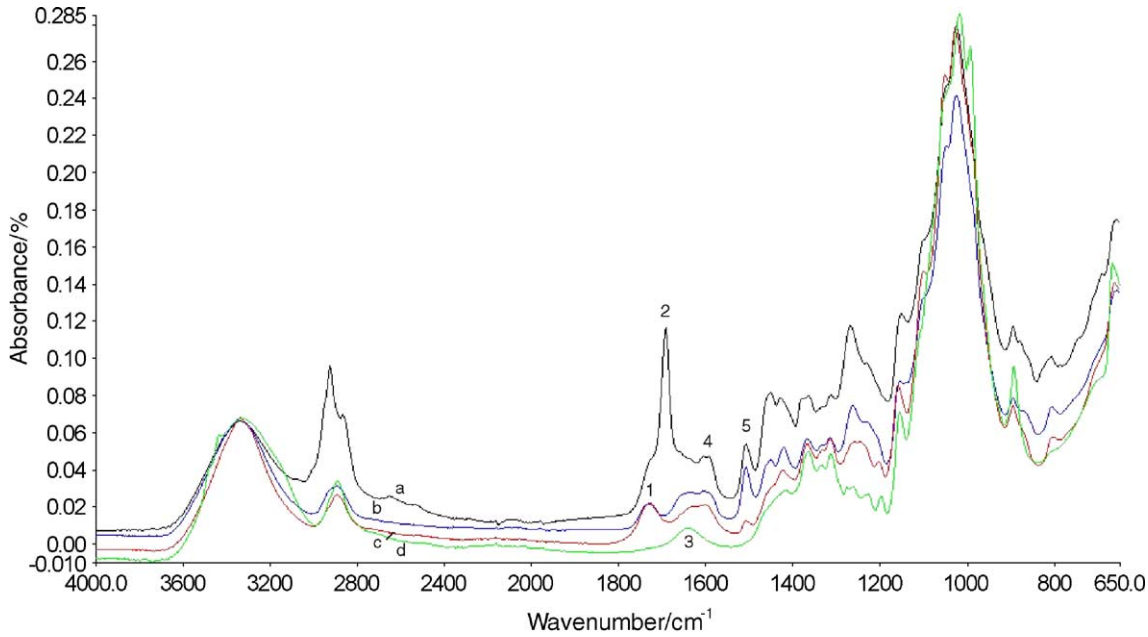

Fig. 1. IR spectra in the different stages of the chemical treatment when using solvent extraction to isolate α-cellulose: a, untreated wholewood sample; b, the sample after solvent extraction; c, after acidified $NaClO_2$ step; d, after NaOH step. Labelled bands are: 1. hemicellulose; 2. resin; 3. linked water; 4. lignin; 5. lignin (Harrington et al., 1964).

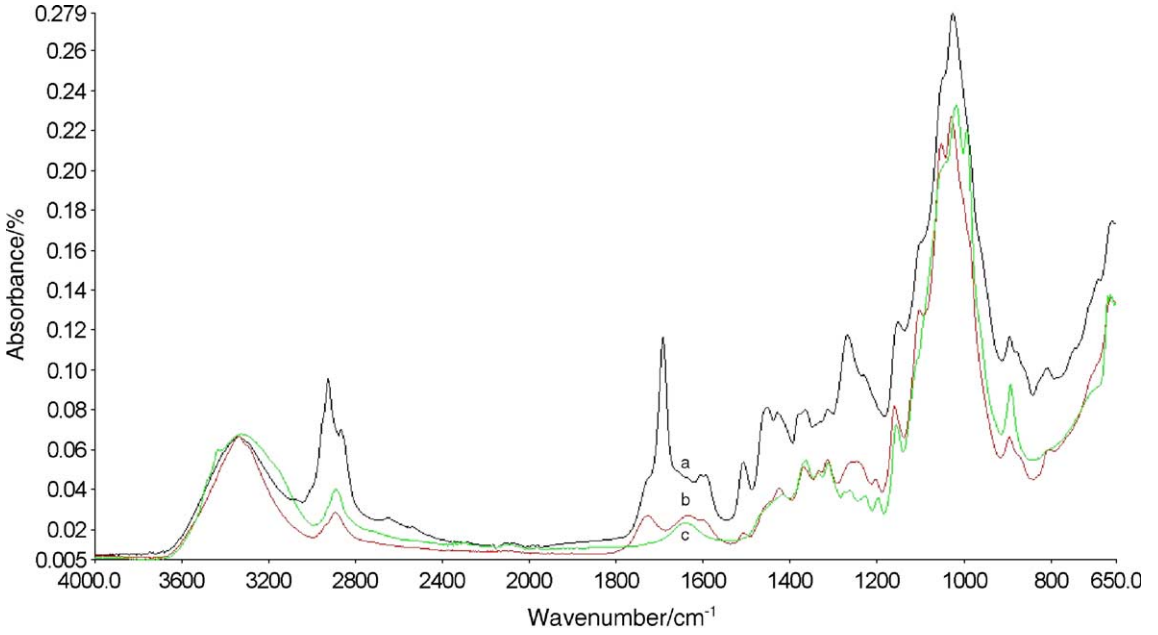

Fig. 2. IR spectra in the different stages of the chemical treatment when solvent extraction is not used: a, untreated wholewood sample; b, the sample after acidified $NaClO_2$ step; c, after NaOH step.

NaOH. There were no traces of the functional groups of resin in the spectra of α-cellulose. This can be seen in Fig. 3 where the α-cellulose prepared with and without the separate resin extraction are compared against Fluka α-cellulose. The same result was found on the other three whole wood samples.

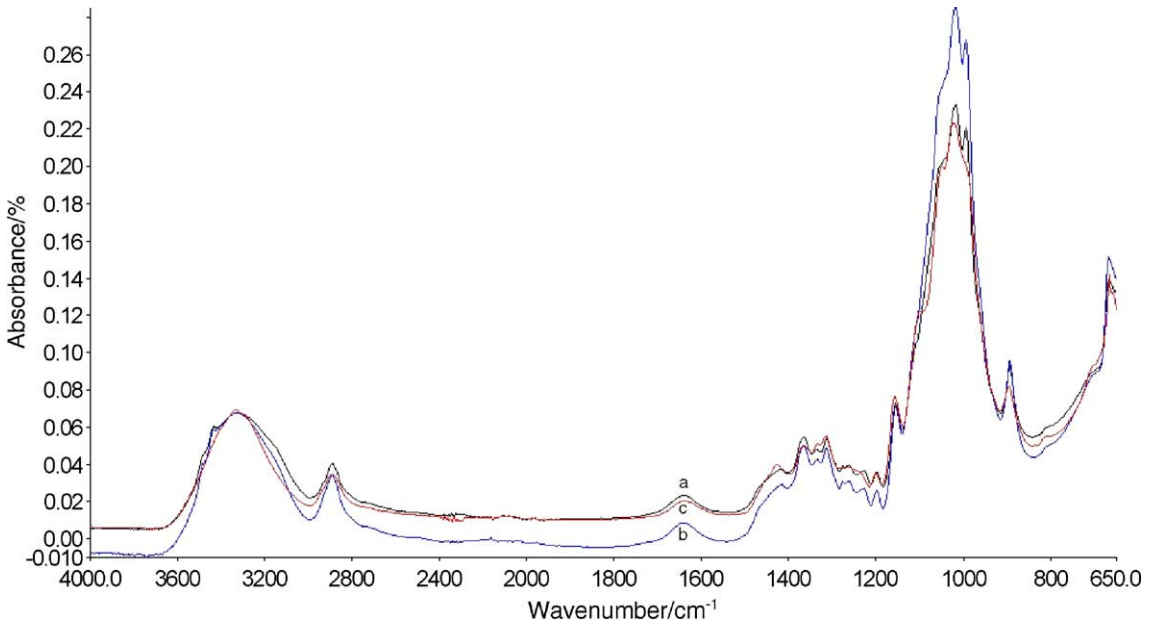

Fig. 3. IR spectra of α-cellulose samples: a, with solvent extraction; b, without solvent extraction; c, Fluka α-cellulose.

From Fig. 3 also the efficacy of the extraction method can be observed. The infrared spectra of two samples of α-cellulose extract from the same wood sample are almost identical to that of a standard α-cellulose (ex. Fluka). This is not the case with the rapid cellulose extraction method developed by Brendel et al. (2000), which shows additional peaks in the infrared spectra of the prepared cellulose.

The results of the analysis of stable isotopes of carbon, oxygen and hydrogen are presented in the Table 1. A *t*-test analysis of the carbon isotope results from the four samples revealed no significant differences between treatments ($P>0.05$) with the exception of the carbon isotope analyses from sample 4 ($P=0.003$). This difference is not believed to reflect the presence of any systematic offset between the two treatments but the very small standard error of the individual means, which fall well within the typical analytical uncertainty of the method (0.1‰ for carbon). If there had been any resins left in the cellulose isolated without the separate stage, this would have shown as lower $\delta^{13}C$ value. This is because resins, which comprise as much as 10% of the total dry matter of wood, are depleted by up to 6‰ in ^{13}C relative to cellulose (Schmidt and Gleixner, 1998; Nuopponen et al., 2004). As far as values of $\delta^{18}O$ and $\delta^{2}H$ are concerned, the small number of replicates precludes detailed statistical analysis. For these isotopes, however, the mean isotope ratios from the two treatments fall generally within the envelope of the standard deviations and also the typical analytical uncertainties of the methods (0.3‰ for oxygen and 2‰ for hydrogen); hence the results do not indicate an isotopic difference between samples obtained with and without resin extraction. Our findings support the results in Hoper et al. (1998), where the carbon isotope values of holocellulose were indistinguishable regardless whether or not samples were treated with ethanol–chloroform before oxidation. This observation, however, was not discussed by the authors.

3.2. Isotopic homogeneity within the cellulose sample

Some of the carbon and oxygen isotope ratios show relatively high standard deviations independent of the treatment used. This is likely to have been caused by isotopic inhomogeneities reflected in the small sample sizes used for isotopic analysis of these elements, i.e. the sample taken for isotopic analysis is not representative of the bulk cellulose sample. It seems that in order to have a representative sample for carbon and oxygen isotopic analysis, especially when the material consists of several annual rings or a pooled sample from several trees, α-cellulose needs to be ground to small particle size in order to ensure that the whole sample is isotopically homogeneous (Borella et al., 1998). This is supported by the relatively low standard deviation in isotope results of the sample 4, which was powdered rather than ground like the other three samples.

3.3. Linked water of α-cellulose

Oxygen isotope ratios of α-cellulose in oak extracted using ^{18}O-enriched water ($\delta^{18}O=29.6‰$) and normal de-ionized water ($\delta^{18}O=-6.9‰$) had $\delta^{18}O$ values of $29.41\pm0.09‰$ ($n=3$) and $29.55\pm0.11‰$ ($n=3$) respectively. There is therefore no evi-

Table 1
Stable isotope ratios ($\pm1\sigma$) of α-cellulose (carbon and oxygen) and nitrocellulose (hydrogen) extracted from samples of Scots pine (*Pinus sylvestris*) with and without solvent extraction of resins

Sample[a]	$\delta^{13}C$ (‰) ($n=10$)		$\delta^{18}O$ (‰) ($n=3$)		$\delta^{2}H$ (‰) ($n=2$)	
Pine no.	With resin extraction	Without resin extraction	With resin extraction	Without resin extraction	With resin extraction	Without resin extraction
1	-24.85 ± 0.17	-24.98 ± 0.24	26.97 ± 0.52	27.03 ± 0.19	-106.3 ± 1.7	-107.3 ± 1.9
2	-25.30 ± 0.20	-25.38 ± 0.19	27.72 ± 0.22	28.17 ± 0.02	-87.2 ± 1.8	-87.1 ± 2.3
3	-24.39 ± 0.11	-24.36 ± 0.32	27.92 ± 0.05	28.10 ± 0.10	-87.4 ± 1.7	-89.5 ± 0.8
4	-23.77 ± 0.13	-23.59 ± 0.08	26.68 ± 0.08	26.74 ± 0.19	-98.3 ± 1.9	-100.7 ± 0.5

Values represent means of replicate analysis.
[a] Sample 1: tree from Northern Finland; Sample 2: tree from UK (heartwood); Sample 3: tree from UK (sapwood); Sample 4: tree from Kola Peninsula (sub-fossil).

dence that the water enriched in ^{18}O influenced the $\delta^{18}O$ value of the cellulose extracted using it. It would seem that either water molecules are not exchanged with linked water molecules in cellulose during the chemical treatments, or that the actual amount of linked water observed in the IR spectra is so small that it does not have a measurable effect on the isotope ratio. It should be noted that the intensity of a band in IR spectra is proportional to the square of the change of the dipole movement during the vibration causing the bands related to water to be disproportionately strong. A third possibility would be that vacuum drying under the described conditions adequately removed any water that might have been exchanged.

4. Conclusions

Based on both the ATR/IR and stable isotope (H, C and O) analysis we conclude that there is no requirement for a solvent extraction stage prior to the separation of α-cellulose from pines for stable isotopic analyses. However, where different species are analysed we would recommend an initial testing of the need of this stage prior to its wider application. We also conclude that water used during the separation procedure does not influence the measured value of $\delta^{18}O$ of the resulting α-cellulose. We also highlight the importance of ensuring isotopic homogeneity within samples. For determination of $\delta^{13}C$ and $\delta^{18}O$, this requires ensuring that the cellulose of very small particle size of sub-mg quantities are removed for isotopic analysis.

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References

Alexander, W.J., Mitchell, R.L., 1949. Rapid measurement of cellulose viscosity by the nitration method. Anal. Chem. 21 (12), 1497–1501.

Anderson, W.T., Bernasconi, J.A., McKenzie, J.A., Saurer, M., Schweingruber, F., 2002. Model evaluation for reconstructing the oxygen isotopic composition in precipitation from tree ring cellulose over the last century. Chem. Geol. 182, 121–137.

Barbour, M.M., Andrews, T.J., Farquhar, G.D., 2001. Correlations between oxygen isotope ratios of wood constituents of *Quercus* and *Pinus* samples from around the world. Aust. J. Plant Physiol. 28, 335–348.

Barbour, M.M., Walcroft, A.S., Farquhar, G.D., 2002. Seasonal variation in $\delta^{13}C$ and $\delta^{18}O$ of cellulose from growth rings of *Pinus radiata*. Plant Cell Environ. 25, 1483–1499.

Becker, B., Kromer, B., Trimborn, P., 1991. A stable-isotope tree-ring timescale of the Late Glacial/Holocene boundary. Nature 353, 647–649.

Berninger, F., Sonninen, E., Aalto, T., Lloyd, J., 2000. Modelling ^{13}C discrimination in tree rings. Glob. Biochem. Cycles 14, 213–223.

Borella, S., Leuenberger, M., Saurer, M., Siegwolf, R., 1998. Reducing uncertainties in $\delta^{13}C$ analysis of tree rings: pooling, milling and cellulose extraction. J. Geophys. Res. 103, 19519–19526.

Brendel, O., Iannetta, P.P.M., Stewart, D., 2000. A rapid and simple method to isolate pure alpha-cellulose. Phytochem. Anal. 11, 7–10.

Browning, B.L., 1967. Methods of Wood Chemistry. John Wiley and Sons, New York. 384 pp.

Farquhar, G.D., O'Leary, M.H., Berry, J.A., 1982. On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol. 9, 121–137.

Gray, J., Song, S.J., 1984. Climatic implications of the natural variations of D/H ratios in tree ring cellulose. Earth Planet. Sci. Lett. 70, 129–138.

Green, J.W., 1963. Wood cellulose. In: Whistler, R.L. (Ed.), Methods in Carbohydrate Chemistry, vol. III. Academic Press, pp. 9–21.

Harrington, K.J., Higgins, H.G., Michell, A.J., 1964. Infrared spectra of *Eucalyptus regnans* F. Muell and *Pinus radiata* D. Don. Holzforschung 18 (4), 108.

Helle, G., Schleser, G.H., 2004. Beyond CO_2-fixation by rubisco—an interpretation of the $^{13}C/^{12}C$ variations in tree rings from novel intra-seasonal studies in broad-leaf trees. Plant Cell Environ. 27, 367–380.

Hiller, A., Boettger, T., Kremenetski, C., 2001. Medieval climate warming recorded by radiocarbon dated alpine tree-line shift on the Kola Peninsula/Russia. Holocene 11, 491–497.

Hoper, S.T., McCormac, F.G., Hogg, A.G., Higham, T.F.G., Head, M.J., 1998. Evaluation of wood pretreatments on oak and cedar. Radiocarbon 40, 45–50.

Knöller, K., Boettger, T., Haupt, M., Weise, S.M., in preparation. $\delta^{2}H$ measurement by continuous flow pyrolysis/isotope ratio mass spectrometry of cellulose nitrate prepared from tree ring series.

Leavitt, S.W., Danzer, S.B., 1993. Method for batch processing small wood samples to holocellulose for stable carbon isotope analysis. Anal. Chem. 65, 87–89.

Loader, N.J., Switsur, V.R., Field, E.M., 1995. High-resolution stable isotope analysis of tree rings: implications of

"microdendroclimatology" for palaeoenvironmental research. Holocene 5, 457–460.

Loader, N.J., Robertson, I., Barker, A.C., Switsur, V.R., Waterhouse, J.S., 1997. An improved technique for the batch processing of small wholewood samples to α-cellulose. Chem. Geol. 136, 313–317.

Loader, N.J., Robertson, I., McCarroll, D., 2003. Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree-rings. Palaeogeogr. Palaeoclimatol. Palaeoecol. 196, 395–407.

Long, A., Arnold, L.D., Larry, D., Damon, P.E., Lerman, J.C., Wilson, A.T., 1979. Radial translocation of carbon in Bristlecone Pine. In: Berger, R., Suess, H.E. (Eds.), Radiocarbon Dating, Proceedings of the Ninth International Conference on Radiocarbon Dating, Los Angeles and La Jolla, 1976University of California Press, Berkeley. 532–537 pp.

McCarroll, D., Loader, N.J., 2004. Stable isotope is tree rings. Quat. Sci. Rev. 23, 771–801.

McCarroll, D., Pawellek, F., 2001. Stable carbon isotope ratios of *Pinus sylvestris* from northern Finland and the potential for extracting a climate signal from long Fennoscandian chronologies. Holocene 11, 517–526.

Mayr, C., Frenzel, B., Friedrich, M., Spurk, M., Stichler, W., Trimborn, P., 2003. Stable carbon- and hydrogen-isotope ratios of subfossil oaks in southern Germany: methodology and application to a composite record for the Holocene. Holocene 13, 393–402.

Moore, A.K., Owen, N.L., 2001. Infrared spectroscopic studies of solid wood. Appl. Spectrosc. Rev. 36, 65–86.

Nuopponen, M., Willför, S., Jääskeläinen, A.-S., Sundberg, A., Vuorinen, T., 2004. A UV resonance Raman (UVRR) spectroscopic study on the extractable compounds of Scots pine (*Pinus sylvestris*) wood: Part I. Lipophilic compounds. Spectrochim. Acta, A 60, 2953–2961.

Pandey, K.K., 1999. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. J. Appl. Polym. Sci. 71, 1969–1975.

Pandey, K.K., Pitman, A.J., 2003. FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. Int. Biodeterior. Biodegrad. 52, 151–160.

Robertson, I., Switsur, V.R., Carter, A.H.C., Barker, A.C., Waterhouse, J.S., Briffa, K.R., et al., 1997. Signal strength and climate relationships in the $^{13}C/^{12}C$ ratios of tree ring cellulose from oak in east England. J. Geophys. Res. 102 (D16), 19507–19516.

Roden, J.S., Lin, G., Ehleringer, J.R., 2000. A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. Geochim. Cosmochim. Acta 64, 21–35.

Saurer, M., Aellen, K., Siegwolf, R., 1997. Correlating $\delta^{13}C$ and $\delta^{18}O$ in cellulose of trees. Plant Cell Environ. 20, 1543–1550.

Scheirs, J., Camino, G., Tumiatti, W., 2001. Overview of water evolution during the thermal degradation of cellulose. Eur. Polym. J. 37, 933–942.

Schmidt, H.-L., Gleixner, G., 1998. Carbon isotope effects on key reactions in plant metabolism and ^{13}C-patterns in natural compounds. In: Griffiths, H. (Ed.), Stable Isotopes. BIOS Scientific Publisher, Oxford, pp. 13–25.

Sheu, D.D., Kou, P., Chiu, C.-H., Chen, M.-J., 1996. Variability of tree-ring $\delta^{13}C$ in Taiwan fir: growth effect and response to May–October temperatures. Geochim. Cosmochim. Acta 60, 171–177.

Switsur, R., Waterhouse, J., 1998. Stable isotopes in tree ring cellulose. Stable Isotopes, Integration of Biological, Ecological and Geochemical Processes. BIOS Scientific Publishers Ltd. 303–321 pp.

Tschamler, H., Kratzl, K., Leutner, R., 1953. The ultra-red spectrum of microscopic wood sections and several model substances. Mikroskopie 8, 238–246.

Waterhouse, J.S., Switsur, V.R., Barker, A.C., Carter, A.H.C., Robertson, I., 2002. Oxygen and hydrogen isotope ratios in tree rings: how well do models predict observed values? Earth Planet. Sci. Lett. 201, 421–430.

Wilson, A.T., Grinsted, M.J., 1977. $^{12}C/^{13}C$ in cellulose and lignin as palaeothermometers. Nature 265, 133–135.