¹⁴C IN EXTRACTIVES FROM WOOD

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ABSTRACT. Two *Pinus aristata* samples submitted by C W Ferguson were separated in different fractions, as was done earlier with *Pinus silvestris* L from Sweden, to yield different fractions for studies of the pretreatment. One sample in this new series consisted of heartwood and the second of sapwood.

The treatment performed in the radiocarbon laboratory involved an acid treatment by boiling, washings, an hydroxide treatment at 80°C, washings and, finally, another acid treatment before being dried before the combustion. The sodium-hydroxide treatment was repeated to yield at least two soluble and two insoluble fractions.

The treatment performed by the wood chemists involved extractions with ethanol-benzene and water. The remaining wood was dated but was also used for the production of holocellulose. The extractives were partitioned between ethyl ether and water and that from the older wood was used for the isolation of neutrals, acids, and phenols. In all, 19 fractions of these two wood samples were dated.

It is confirmed that a treatment for 30 min in sodium hydroxide at 80°C is not sufficient to remove the extractives from the heartwood or the sapwood. A treatment at 80°C overnight with 1 percent NaOH yielded a sample from the older wood with a ¹⁴C content in good agreement with the results predicted from the curve presented by Stuiver (1978). The final statistical uncertainty in the present investigation was ca 4‰. The younger wood yielded results indicating a lower activity than that given by Stuiver.

INTRODUCTION

Wilson (1961) examined heartwood of *Pinus aristata* and found that the extractives had the same activity as the cellulose, but he used the outer heartwood rings from a tree felled in 1959. The outer 11 rings were sapwood. When the heartwood was formed from the sapwood, between 1957 and 1959, the radiocarbon activity in the southern hemisphere was only between 5 and 15 percent above the natural level around 1947. Any possible effect was, thus, difficult to detect.

Wilson, Gumbley, and Speddin (1963) presented evidence for a difference in activity between acetone-extracted material and cellulose derived from sapwood dating from 1953-54 and later, clearly indicating that there was bomb-produced radiocarbon in the resin. Jansen (1970) gave further evidence for a difference in the apparent ages of cellulose and resin from tree rings. What he indicated as "cellulose" should have been quoted as "extracted wood", since it still contained lignin. Similar results were given in 1969 in the discussions at the Nobel Symposium in Uppsala (Olsson, 1970, p 271-273). Olsson, El-Gammal, and Göksu (1969), Olsson, Klasson, and Abd-El-Mageed (1972), and Olsson (1979) published the results of some investigations indicating a radial transport.

Berger (1973) tried to discover whether there was any diffusion of tree sap into older rings by injecting ¹⁴C-labelled sucrose. He did not see any increased activity because of this injection. Berger (1970) also failed to detect the transfer of carbon compounds from the outer rings to the inner rings. A similar conclusion was reached by Tans, de Jong, and Mook (1978), studying the 1962-1963 transition.

Libby and Pandolfi (1976) used the total wood for their isotope-tree thermometer. Wilson and Grinsted (1977) and Long and Lerman (1977)

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demonstrated the variations with temperature of the $\delta^{13}C$ for cellulose and lignin and presented plans for further studies of isotope thermometers, using well-defined components of the wood. Long and others (1979) discussed radiocarbon excess in extractives from heartwood.

Activity calculations

The procedure for the activity measurements is essentially that given by Olsson (1958) and the actual procedure for the calculations is given by Olsson (1966). The changes made later in the program do not affect the basic principles of the calculations. The per mil deviation from the PDB standard of the ^{13}C content is given as $\delta^{13}C$ and is measured by R Ryhage and his staff at the mass-spectrometric laboratory at Karolinska Institutet in Stockholm. The normalized and age-corrected, per mil deviation from the standard of the ^{14}C activity is given by:

$$\Delta = \delta^{14}C - (2\delta^{13}C + 50)\left(1 + \frac{\delta^{14}C}{1000}\right),$$

where $\delta^{14}C$ is the age-corrected, ^{14}C deviation from the standard (95 percent of the activity of the NBS oxalic acid in AD 1950, with a $\delta^{13}C$ value of $-19\%_0$ on the PDB scale). The formula, however, is slightly incorrect (Olsson and Osadebe, 1974; Stuiver and Robinson, 1974). This fact does not alter the conclusions drawn here.

Samples

The samples are bristlecone-pine wood from TRL74-101, kindly submitted by C W Ferguson, Laboratory of Tree-Ring Research, Tucson, Arizona. One of the samples was sapwood, dated between AD 1910 and 1930 and the other heartwood, dated between AD 1835 and 1855. Both samples were, thus, of pre-bomb origin, although the tree was felled in 1974, allowing studies of the movement of bomb-affected material inside the tree. An earlier study of other rings of the same tree was made by Long and others (1979). They used heartwood dating between 1855 and 1875, but part of the tree rings in this interval still consisted of sapwood. Thus, it can be assumed that part of the 1835-1855 wood still consisted of sapwood in 1955 and probably a few years later. In 1974, very little sapwood was present in rings prior to the 1865 ring.

Parts of both samples were given standard pretreatment in the radiocarbon laboratory and parts were separated into different compounds, using organic solvents and thin-layer chromatography.

Standard pretreatment

Part of each sample, 10g, was given the treatment described and illustrated by Olsson (1979, fig 3). To test the NaOH treatment, it was applied in three steps. First, the wood and the hydroxide were heated separately to 80°C before being mixed. The extraction was broken off after 30 min and the wood was repeatedly washed with distilled water and then treated again with the hydroxide, overnight. After rewashing, half of the sample was treated a third time with NaOH. The fractions dissolved by NaOH are called "SOL" and the insoluble frac-

tions are called "INS". For this investigation, the extractives obtained at the initial treatment with hot HCl solution were kept and dated. The procedure and the results of the measurements are given in figure 1. The first step is depicted in the lower part of the figure and the last step in the upper part. An effort has been made to give approximate figures for the yields at the different steps. For convenience, the amount of CO_2 from the initial HCl treatment of the sapwood was chosen as reference. The two insoluble fractions of each sample, added together, yielded the same amount of carbon dioxide, within ca 5 percent, but about nine times more material was extracted from the heartwood than from the sapwood, although most of the extractives from the heartwood did not exhibit any increased activity. The third extraction with the alkali yielded so little material that it was not worth trying to date it. Indeed,
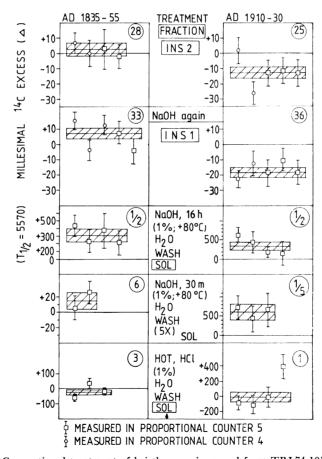
Fig 1. Conventional treatment of bristlecone-pine wood from TRL74-101 and radiocarbon measurements from December 1976 to October 1978. The older sample was heartwood and the younger one, sapwood. Heartwood formation from sapwood probably occurred after 1955 for part of the wood, since it is known that the 1855-75 wood partly contained sapwood in 1974.

some of the dated fractions were diluted more than is normally accepted in the laboratory in order to reach the normal working pressure of the proportional counters. The two insoluble fractions of each sample were not diluted.

Figure 1 shows that the two insoluble fractions of each sample yielded results confirming that the third NaOH extraction was not necessary. The pretreated wood from the period 1835-1855 has an activity which, after age correction, does not differ significantly from the standard activity, whereas the wood from the period 1910-1930 has ca 2 percent less ^{14}C activity after age correction. HCl treatment apparently did not remove any active substances. The first NaOH treatment, lasting half an hour, removed active substances from the sapwood, but the extractives from the heartwood did not show any activity different from the insoluble end products, although almost 10 percent of the sample was removed. The second extraction from both samples yielded a substance with a significantly higher activity than that of the end product. The uncertainty of the values is great, but the values happen to be about the same and the amount of material does not differ very much. For further discussions, a rounded figure of 1 percent for the amount of material extracted from each sample with an excess activity of ca $300\%_0$ for the heartwood and ca $400\%_0$ for the sapwood can be used. If the pretreatment with NaOH had been stopped definitely after half an hour, an error of ca $3\%_0$ would have been introduced. When applied to normal calibration work for periods with slightly varying activities, the observed phenomena mean that 1 percent of material with an excess activity of $30\%_0$ may easily introduce an error of $0.3\%_0$ in the figure of the final excess for the cellulose in the tree rings studied, if the alkali treatment is interrupted after half an hour. A prolonged treatment apparently does not remove more material than is absolutely necessary, at least to judge from this particular bristlecone-pine sample. Indeed, 60 percent of the sample was left as the insoluble fractions.
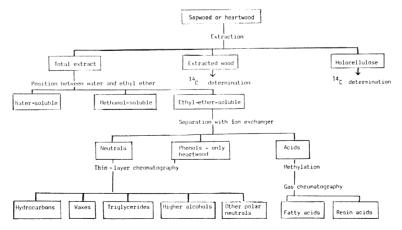

Fig 2. Treatment using organic solvents, applied by a wood chemist.

Wood analysis

Because of the interest shown by Ferguson in the chemistry of bristlecone-pine wood in 1976, when we discussed the results from *Pinus silvestris* (Olsson, 1979). Gunnel Åkerlund, Mo och Domsjö AB, Örnskoldsvik, performed an analysis of the wood. The work was carried out independently, great care being taken that all organic solvents were carefully removed (Jansen, 1973; Olsson and others, 1974). Åkerlund described the experiment reported here (tables 1, 2; figs 2, 3).

Wood chemistry

The extraction was made on ground wood in a Soxhlet extraction apparatus with ethanol-benzene (1:2 v/v) for 20 hours. The extracted wood meal was dried at room temperature and then extracted with water for 20 hours. All the separations of the extractives are given schematically in figure 2.

The extraction solutions were liberated from the solvents under reduced pressure and the final drying was performed in a desiccator over potassium hydroxide. The extract was partitioned between ethyl ether and water. Some material remained undissolved but could be dissolved in hot methanol. The original extract was, thus, divided into extractives soluble in water, methanol, and ethyl ether (table 1).

The water-soluble extractives and the methanol-soluble extractives were not further investigated.

Most of the ethyl-ether-soluble extractives from the sapwood were used for radiocarbon measurements. The rest was fractionated on a DEAE-Sephadex column into neutrals and acids (table 1).

Part of the ethyl-ether-soluble extractives of the heartwood was used for radiocarbon measurements. The rest was fractionated, as for the sap-

TABLE 1

The composition of wood samples from heartwood and
sapwood of bristlecone pine

Compound	Heartwood		Sapwood	
	% of ethyl-ether-soluble	% of wood	% of ethyl-ether-soluble	% of wood
Lignin, % of extracted wood		28.0		29.1
Total extractives		17.31		5.77
Water-soluble extractives		2.01		1.61
Methanol-soluble extractives		1.93		0.36
Ethyl-ether-soluble extractives	100	13.37	100	3.79
Phenols	32.63	4.36	—	—
Acids	50.21	6.71	71.49	2.71
Resin acids	33.36	4.46	17.99	0.68
Fatty acids	16.80	2.25	53.50	2.03
Neutrals	17.15	2.29	28.51	1.08
Hydrocarbons	0.39	0.05	0.68	0.03
Waxes	5.52	0.74	3.14	0.12
Triglycerides	8.88	1.19	14.31	0.54
Higher alcohols	1.37	0.18	3.79	0.14
Other polar neutrals	0.96	0.13	6.59	0.25

wood, into neutrals, phenols and acids (table 1)—the main part being used for radiocarbon measurements and the rest, further analyzed.

Acid fractions were methylated with diazomethane and methyl esters of the acids were analyzed by gas chromatography (table 2).

The phenolic fraction was analyzed by thin-layer chromatography (fig 3). The main part of the fraction (78 percent) was a phenol not earlier investigated at Mo och Domsjö AB. The phenolic fraction was, therefore, methylated with diazomethane and was analyzed by gas chromatography (table 1).

Neutrals were analyzed by thin-layer chromatography (fig 3). The heartwood neutrals contained a new substance, probably a phenol, which ran near to the same point as the triglycerides but was not further investigated. Neutrals were also separated by preparative, thin-layer chromatography into hydrocarbons, waxes, triglycerides, alcohols, and other polar neutrals (table 1).

Part of the extracted wood meal was used for radiocarbon analysis, part for lignin analysis, and part for holocellulose production. The holocellulose was then used for radiocarbon analysis.

Radiocarbon content of compounds separated by wood chemistry

All the radiocarbon measurements are summarized in figure 4. The holocellulose fraction is supposed to be the final end-product yielding the most reliable results. The present mean holocellulose values agree fairly well with those of the end products in the conventional treatment. The extracted wood is also regarded as a reliable product,
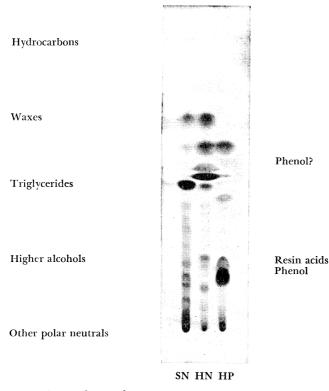
TABLE 2

Relative composition of the acids

Compound*	Acids	Heartwood	Sapwood
	Fatty acids	33.5	74.8
16:0	Palmitic acid	0.93	8.87
17:0 br		0.98	6.61
18:0 br		0.11	6.74
18:0	Stearic	0.22	4.13
18:1	Oleic	4.87	28.61
18:2	Linoleic	10.15	3.81
18:3 5. 9.12	Pinolenic	0.45	1.27
18:3 9.12.15	Linolenic	2.55	+
20:1		0.33	1.11
Unidentified		1.53	+
22:0		3.07	+
23:0		6.76	10.68
	Unidentified	1.65	2.10
	Unidentified		0.89
	Resin acids	66.44	25.2
	Levopimaric/Palustric	7.02	+
	Isopimaric	16.32	4.96
	Abietic	34.67	4.64
	Dehydroabietic *a*	7.20	13.19
	Unidentified	1.23	2.38

* First digits indicate the number of carbon atoms in the chain; second digit denotes the number of double bonds, and the following digits indicate the position of double bond.

since the extractives are removed and the present mean values agree with those of the other end-products, although the statistical uncertainty is still somewhat greater than necessary. New measurements have been initiated, although one purpose of this investigation was to trace the influence of different extractives in contributing to the choice of a proper pretreatment.

The extractives, soluble in ethyl ether, were proven to have increased ^{14}C activity. The excess is slightly larger for the sapwood than for the heartwood.

The extractives from the heartwood could be further separated into several fractions. The three datable extractives have about the same activity. The present mean value is corroborated by the activity of ethyl ether extractives. The small difference may be a statistical spread but may also derive from less active material in the methanol-soluble extractives. Solvents remaining in the ethyl-ether extractives would also lower the activity.

Hydrocarbons

Waxes

Phenol?

Triglycerides

Higher alcohols

Resin acids
Phenol

Other polar neutrals

SN HN HP

SN = Sapwood neutrals
HN = Heartwood neutrals
HP = Heartwood phenols

Fig 3. Thin-layer chromatography on neutrals and phenols from the ethyl-ether-soluble extractives in sapwood and heartwood.

¹³C content

δ¹³C values of the wood after treatment with either NaOH or ethyl ether agree well with the results usually published for *Pinus aristata*. For instance, we calculated a mean value of −22.9‰ from 202 samples dated at the Pennsylvania laboratory. Removal of lignin to yield holocellulose will give a slightly lower negative value of δ¹³C, that is, it will contain more ¹³C than the wood. All extractives are deficient in ¹³C (table 3), except the HCl–H_2O extractives, when compared with the holocellulose. The only significant difference between the two samples is in the ethyl-ether-soluble extractives, the sapwood being more deficient in ¹³C than the heartwood.

CONCLUSION

Wood, dating from the period 1835-1855 should, according to Stuiver (1978), have an activity very close to the standard after age correction. The values presented here indicate the same. The mean value of the two fractions called INS and the two fractions obtained after removal of the extractives in a Soxhlet apparatus is, indeed, (+0.6±2.5)‰. Similarly, sapwood should have a deficiency in ¹⁴C content of ca 10‰. The four values presented here indicate (−9.2±2.5)‰.
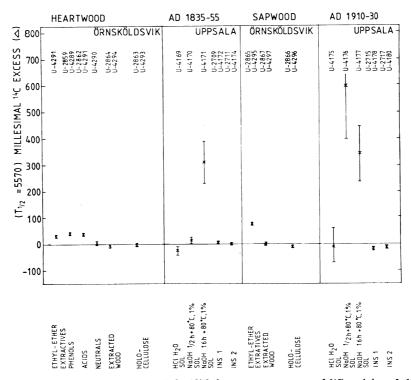

Fig 4. Results achieved up to July 1979 from measurements of ¹⁴C activity of different fractions of two bristlecone-pine samples.

The large excess of ^{14}C in various extractives of the bristlecone-pine must be due to material transported from the outer tree rings to some of the inner rings. This material shows such a high activity that it has, undoubtedly, been affected by nuclear weapon tests. Sapwood is converted into heartwood when ca 100 years old, at least in this particular tree. Thus, it is proven that movement occurs, at least, within the sapwood and that the material with higher activity is locked in, although probably chemically altered in connection with the heartwood formation. The next step would be to examine older wood, which was already heartwood when the nuclear bomb tests started.

Closer scrutiny of the activities of the different fractions (fig 1) and the yield reveals that conventional treatment of heartwood yielded a soluble fraction with $(311\pm80)\%_o$ excess in ^{14}C, which amounted to 0.8 percent of the whole sample, and another NaOH-soluble fraction, with no excess within $\pm15\%_o$, which amounted to 9 percent of the sample. The wood chemistry and the other activity measurements imply that the excess is to be found in the ethyl-ether-soluble extractives, or in 13.37 percent of the sample, and possibly in the methanol-soluble extractives, or in 1.93 percent of the sample. From conventional treatment, it seems as if no excess radiocarbon is present in the water-soluble fraction. Radiocarbon "added" to the heartwood is, thus, equivalent to $\geqslant(250\pm160)\times10^{-5}$ of the total activity of the sample, using data from conventional treatment, and to (420 ± 70) to (480 ± 80), using data from the ethyl-ether extractive, but at least $(450\pm70)\times10^{-5}$, using data from the individual extractives (phenols, acid, and neutrals). Radiocarbon "added" to sapwood is, similarly, equivalent to $\geqslant(450\pm140)\times10^{-5}$ of the total activity of the sample, using data from conventional treatment, and $(290\pm15)\times10^{-5}$ to $(320\pm15)\times10^{-5}$, using data from the ethyl-ether extractive.

TABLE 3

$\delta^{13}C$ values for dated fractions of the TRL74-101 samples. The uncertainty is estimated to be $\pm0.4\%_o$ for these measurements. The statement is based on real deviations observed in repeated measurements.

Fraction	Heartwood 1835-55	Sapwood 1910-30
HCl–H_2O-soluble extractives (boiling HCl, 1%)	−17.4‰	−17.6‰
NaOH-soluble extractives (+80°C, 1%), 30 min	−20.7	−22.2
NaOH-soluble extractives (+80°C, 1%), next 16 hours	−21.5	−20.3
Extracted wood (16½ hours treatment with NaOH)	−21.0	−21.6
Extracted wood (16½ + 16 hours treatment with NaOH)	−21.2	−21.1
Ethyl-ether extractives	−23.0	−26.7
Phenols	−22.7	
Acids	−24.0	
Neutrals	−24.5	
Extracted wood (ethanol-benzene)	−20.3	−21.3
Holocellulose	−19.0	−19.5

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