width (W) for the entire section of tree used for production of the climate curve[1]. Values of L/W ranged from 0.05 for the largest value of W to 0.32 for the smallest W. Further, $^{18}O/^{16}O$ isotopic analyses were carried out on early and late wood from a number of selected rings from the tree. (These were necessarily rings having larger values for W due to difficulties associated with the sampling of narrow rings and insufficiency of material for analysis.) The range of values found for the difference in isotopic composition between early and late wood ($\delta E - \delta L$) was found to be 0.5 to 0.8‰.

Thus using equation (1) as stated by Wigley *et al.* with values of $\delta L - \delta E = -0.8$‰ and $L/W = 0.32$ the maximum variation in δW due to the term dependent on total ring width is found to be 0.26‰. Thus the maximum contribution to the variation is estimated at somewhat less than 10% of the total variation observed (3‰) in a 100-yr-old tree. Furthermore, by taking 5-yr groups of rings this effect will tend to be minimised since the tree in question contained few narrow rings and the effect is much less significant for wider rings (L/W of the order 0.05). In general, therefore, we expect the effect to be considerably less than the 10% suggested above.

The measured values of tree ring width (W) for the Edmonton spruce, after growth curve corrections were made, show little or no correlation with mean annual temperatures. We conclude that in this case, the contribution of ring-width effects to variation in $\delta^{18}O$ of the cellulose is minimal. This finding, however, does not detract from the potential significance of the effect for other trees in other climate zones. Care should be taken to select trees which are 'complacent', that is, those with ring widths showing little annual variation. (This is in contrast to the requirements of those establishing climate curves from ring-width measurements[2].) The second possible effect of ring-width variation, that of introduction of a sampling error, arises because, when sampling 5-yr groups of rings[1], no attempt was made to normalise the amount of wood contributed by each of the rings to the material subsequently analysed. When calculating mean annual temperatures for each 5-yr period, however, an arithmetic mean was used, implying equal contribution from each ring and hence implying equal ring width throughout the 5-yr group. To estimate the error introduced by this procedure, we measured total ring widths for the entire tree. Using mean annual temperature (T) from climate records we calculated the expected isotopic composition of the cellulose in each ring using the relation $\delta^{18}O = 1.3T + 20.5$ (‰) (ref. 1). The mean $\delta^{18}O$ for each 5-yr group of rings was then calculated first using

Table 1 Comparison of ring-width weighted mean, non-weighted mean and measured $\delta^{18}O$ values for cellulose extracted from 5-yr groups of tree rings

Period	$\delta^{18}O$ (‰) weighted mean	$\delta^{18}O$ (‰) unweighted mean	$\delta^{18}O$ (‰) measured
1894–99	23.4	23.5	23.4
1900–04	24.2	24.4	24.1
1905–09	24.7	24.6	24.7
1910–14	24.6	24.6	24.7
1915–19	24.0	24.0	24.1
1920–24	23.8	24.0	24.2
1925–29	23.7	23.5	23.6
1930–34	24.6	24.7	24.7
1935–39	23.2	23.3	22.9
1940–44	24.9	24.6	24.5
1945–49	23.8	23.7	23.3
1950–54	23.7	23.3	23.3
1955–59	24.4	24.5	24.5
1960–64	25.1	25.3	25.2
1965–68	23.5	23.7	24.0

the ring-width data as a means of weighting the $\delta^{18}O$ contribution of each ring and second, assuming equal contributions for each ring in a given 5-yr group. The calculated weighted and unweighted means, together with measured values are shown in Table 1. The largest difference between weighted and unweighted calculated value is 0.4‰, approximately twice the estimated precision of the measured data (± 0.2‰). It seems that errors introduced elsewhere in the procedures used, are usually equal to or greater than those due to sampling errors. It should be pointed out, however, that trees showing marked variability of ring width with climate may well present sampling problems of this kind.

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LIBBY AND PANDOLFI REPLY—Pandolfi has shown that isotope variations in tree rings are correlated with climate variations local to the trees. Wigley *et al.* speculate that isotope variations in tree rings may be correlated with ring widths. Then if they are correct, it must follow that ring widths are correlated with climate.

We consider that the master chronologies of ring widths from the European tree laboratories will not correlate with local climate variations. We found no correlation between isotope measurements for a German oak and B. Huber's master oak ring chronology.

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Amide nitrogen is unlikely to be a proton acceptor

IN their article on the mechanism of catalysis of acid proteases and extended to proteases generally, James *et al.*[1] make several statements to the effect that a tyrosine donates its proton to the amide nitrogen of the scissile bond in a peptide substrate. This mechanistic idea is similar to that suggested for hydrolysis of peptide substrates by carboxypeptidase[2,3]. But the peptide nitrogen is not the basic site in an amide bond and cannot accept a proton, a hydrogen bond, or a metal ion in the ground state of an enzyme–substrate complex[4]. This restriction holds with even more force when the amide carbonyl oxygen interacts with a proton or a metal ion, as is thought to be the case with carboxypeptidase.

There are many data to support the view that the basic site in an amide bond is the carbonyl oxygen so that either a proton[5,8] or a metal ion[7,8] associates at that atom. These conclusions are supported by theoretical calculations which indicate a strong preference for protonation of amides at the carbonyl oxygen[9,10]. Unless there is enormous strain associated with substrate binding, the earliest stage in a hydrolysis mechanism during which an amide nitrogen may become a proton, hydrogen bond, or metal ion acceptor is concerted with nucleophilic attack at the amide carbon which results in both the carbon and nitrogen of the amide bond taking on tetrahedral character[4]. At this point the nitrogen has lost its amide character and has become an amine nitrogen. This principle seems to have been accepted in a recent discussion of the mechanism of thermolysin which is thought to be similar to that of carboxypeptidase[11]. Though the authors of the acid protease paper[1] may feel that they did not violate this principle in their own minds, their statements coupled with proposed mechanisms in the literature cited are apt to mislead many readers.

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