ionized constituents seem to be ruled out because of the rapid recombination rates required for such ions and because there are no observed ions at $m/e = 90$. This constituent also exhibits its peak concentration in the E_s-layer (Fig. 1), which implies a high probability that it is atomic in structure. Instrumental contaminants and irregularities do not appear likely since 45+ reappears at the same altitude in the downleg data. The above indicate an ion enhancement of the order of 100 times the normal relative abundance of Sc seen either in chondrites or in the earth's crust. This may be a property of the cometary debris.

The measurement of metallic ions in the upper atmosphere during the period of the β Taurids meteor shower suggests that extraterrestrial debris can contain enriched abundances of trace constituents not predictable from the cosmic abundance of these constituents. The enhanced densities of such constituents are still quite small and probably below the threshold of detection by other techniques, such as ground-based optical sensing. Hence, in situ measurements of the ion composition of the atmosphere during and after the entry of significant amounts of extraterrestrial debris offer a unique opportunity to analyze and study such material.

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Lignification in Trees: Indication of Exclusive Peroxidase Participation

Abstract. *Syringaldazine did not turn purple on cross sections of tree branches or saplings or on cambial tissue cultures unless hydrogen peroxide was added; this indicated the absence of laccase but presence of peroxidase in lignifying cells. Peroxidase, therefore, apparently is the only enzyme that polymerizes* p*-coumaryl alcohols to lignin in trees.*

The occurrence of coniferin in the sap of many gymnosperms helped to establish a theory that lignin is formed from its aglycon, coniferyl alcohol. Testing this theory, Freudenberg *et al.* (*1*) used indican to detect a β-glucosidase in the region of lignification in xylem cells near the cambium of *Araucaria excelsa*. The glucosidase released indoxyl that autoxidized to indigo, indicating the amount and distribution of β-glucosidase in the wood: a weak color in cells near the cambium intensified inward through thickening lignifying cells to a breakoff point in mature xylem tissue.

However, the enzyme involved in the next stage of lignification, the polymerization of *p*-hydroxycinnamyl alcohol precursors to lignin, remained unknown.

The Freudenberg group used a fungal phenol oxidase, later shown to be laccase (*p*-diphenol : O_2 oxidoreductase, E.C. 1.10.3.2), to make biosynthetic lignins (dehydrogenation polymer) in vitro from coniferyl alcohol alone or its mixtures with sinapyl and *p*-coumaryl alcohols (*2*, *3*). It was thought that laccase catalyzed an analogous polymerization to produce lignin in wood (*2*, *4*).

The incidence of laccase in fungi is widespread. However, lack of information on its distribution in higher plants, well-documented data on peroxidases in many lignified species, and the ability of peroxidase and H_2O_2 to transform *p*-coumaryl alcohols into lignin-like

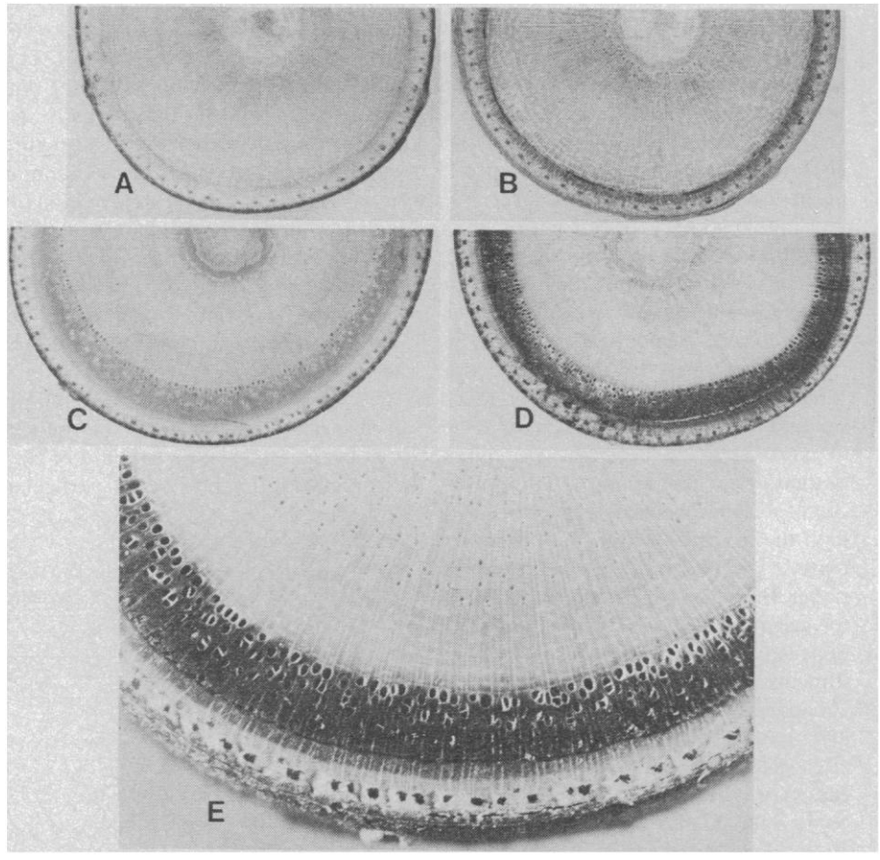
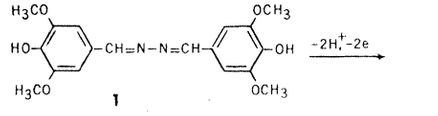


Fig. 1. Freshly cut surfaces of green ash sapling stems swabbed with dilute aqueous alcoholic furoguaiacin: (A and C) before adding H_2O_2; (B, D, and E) after adding dilute H_2O_2 in ether.

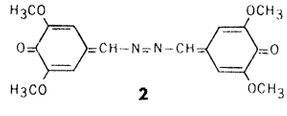
polymers just as well as laccase and air (*2, 4*) induced Higuchi (*4*) and Lyr (*5*) to favor peroxidase (donor : H_2O_2 oxidoreductase, E.C. 1.11.1.7) as the lignifying mediator in plants.

However, Wardrop and Bland (*6*) found that the distribution of peroxidase in pine seedlings, measured by the production of a blue color with benzidine and H_2O_2, was actually complementary to the distribution of lignin in the cell wall. Koblitz and Koblitz (*7*) therefore looked for cytochrome oxidase (E.C. 1.9.3.1) in the zone of lignification by using Nadi reagent, and ascribed to it responsibility for the phenol oxidative lignification reaction. Later, Lipetz (*8*) determined that peroxidase interfered with this reagent, and reinstated peroxidase as the most probable phenol dehydrogenating enzyme in plants.

We have applied syringaldazine to various tree species to finally resolve the lignification oxidase question. Syringaldazine (**1**) is a pale-yellow crystalline compound prepared easily from syringaldehyde and hydrazine (*9*). With laccase, phenol oxidation of (**1**) occurs with atmospheric oxygen acting as hydrogen acceptor to give the highly conjugated, intensely purple tetramethoxyazo-*p*-methylenequinone (**2**).



1



2

Peroxidase plus H_2O_2 produces the same effect. No color change occurs with tyrosinase (*o*-diphenol : O_2 oxidoreductase, E.C. 1.10.3.1) or cytochrome oxidase.

In early spring, numerous samples of branches and saplings were collected from angiosperms that were beginning to show bud expansion (green ash, *Fraxinus pennsylvanica* Marsh.; sugar maple, *Acer saccharum* Marsh.; quaking aspen, *Populus tremuloides*, Michx.; eastern cottonwood, *Populus deltoides* Bartr.; European mountain ash, *Sorbus aucuparia* L.; lilac, *Syringa vulgaris* L.; and apple, *Malus* sp.) and gymnosperms that were beginning to elongate (eastern white pine, *Pinus strobus* L.; white spruce, *Picea glauca* (Moench) Voss; Norway spruce, *Picea abies* (L.) Karst.; balsam fir, *Abies balsamea* (L.) Mill.; and yew, *Taxus cuspidata* Sieb. et Zucc.). Fresh samples 150 to 200 mm in diameter were sectioned transversely on a freezing microtome.

Application of one or two drops of 0.1 percent solution of syringaldazine in ethanol to cross sections of freshly microtomed surfaces of sample stubs did not produce even the faintest trace of pink or red coloration to indicate the presence of laccase. However, when one or two drops of 0.03 percent aqueous H_2O_2 were added to the section or end surface, an intense purple ring formed almost immediately in the xylem tissue adjacent to the cambium. Within minutes, the color spread inward through the medullary rays, then over the whole surface, probably by diffusion of reagents and of enzyme solution from the wounded tissue. To observe and record the initial staining zone, it was therefore preferable to use stub surfaces rather than sections, and to blot off excess azine or peroxide with tissue or filter paper. Color photographs of the stained surfaces could thus be easily obtained. Reproduction of typical photographs of this reaction with green ash have appeared (*10*).

It was difficult to obtain staining intense enough for good contrast on black-and-white film. The location of the enzyme could, however, be recorded by using a similar technique with another reagent. Furoguaiacin, the active principle of the α-guaiaconic acid fraction of gum guaiac, is also dehydrogenated by phenol oxidases (laccase or tyrosinase plus air, or peroxidase plus H_2O_2) to yield a blue bismethylenequinone pigment, guaiacum blue (*11*). Transections of samples treated with an aqueous alcoholic solution of furoguaiacin remained colorless, indicating absence of both laccase and tyrosinase. When H_2O_2 was added, a blue stain appeared in the regions in which the purple color occurred with syringaldazine. Again, some color diffused throughout the surface after a few minutes; the diffusion was retarded by using H_2O_2 in ether rather than in water. Figure 1 shows transections treated with furoguaiacin before (Fig. 1, A and C) and after (Fig. 1, B, D, and E) peroxide addition. The stem shown in Fig. 1, A and B, was cut when the buds were beginning to swell (before xylem production commenced). The main staining due to peroxidase is at the cambium and in the phloem. The twig shown in Fig. 1, C and D, and in greater magnification in Fig. 1E was taken from the same tree 3 weeks later. The darkest area is still at the cambium, but the color now extends inward toward the mature xylem and breaks off abruptly at the edge of the previous year's growth. The color intensity lessens progressively inward from cambium to mature springwood. Early springwood vessels in the current growing season (see Fig. 1E) no longer show peroxidase activity, reflecting their completed differentiation. Ray cells of both years also show no peroxidase activity although their cytoplasm is still alive. However, the associated paratracheal parenchyma in the previous year's growth exhibited weak peroxidase activity in some other ash samples examined.

In general, staining varied in intensity and breadth depending on the species and the amount of differentiated tissue (primarily xylem); this reflected the degree of growth in each specimen. Usually angiosperms gave more intense, broader colorations than gymnosperms; lilac and mountain ash reacted particularly strongly. The initial coloration occurred on both phloem and xylem tissues adjoining the cambium. No specimens gave any colorations with syringaldazine alone or furoguaiacin alone; in every sample, rapid staining took place only after hydrogen peroxide was added. It therefore seems certain that the phenol oxidase in the zone of incipient lignification is exclusively peroxidase.

Syringaldazine, furoguaiacin, and coniferyl alcohol are all unaffected by beef heart cytochrome oxidase; thus, this enzyme is eliminated from consideration as the oxidase catalyzing the phenol oxidations that lead to lignin.

Analogously, callus cultures grown from cambium of green ash, *F. pennsylvanica* Marsh., quaking aspen, *P. tremuloides* L., and pin oak, *Quercus palustris* Muenchh., showed no coloration when treated with syringaldazine or furoguaiacin alone, but abundant purple or blue stains appeared when dilute H_2O_2 was added. If fed suitable growth hormones, these tissues can form up to 50 percent of their dry weight of lignin-like material (*12*). It is again apparent that the phenol oxidase responsible for the dehydrogenative polymerization of lignin precursors is exclusively peroxidase.

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High-Pressure Polymorph of Thulium: An X-ray Diffraction Study

Abstract. *An x-ray diffraction study of thulium at room temperature and high pressure by means of a diamond-anvil press has shown that thulium transforms from a hexagonal close-packed structure to the samarium type, as other rare-earth elements (gadolinium, terbium, dysprosium, and holmium) do. Unlike the other rare-earth elements, thulium (hexagonal close-packed) has an axial ratio* (c/a) *that is independent of pressure within experimental error and the transition is reversible. The transition occurs with increasing pressure in the range of 60 to 116 kilobars. The lattice parameters of the samarium-type phase of thulium at about 116 kilobars are* a = *3.327 ± 0.005 angstroms and* c = *23.48 ± 0.04 angstroms, and the volume change at the transition is estimated to be − 0.5 percent of the volume of the hexagonal close-packed phase at the transition.*

In a recent study of the correlation between the pressure-induced phase transformations and the periodicity of density for solid elements, one of us (L. L.) has proposed an empirical relation which serves as a better basis for predicting high-pressure polymorphism in solid elements than crystal structure does (*1*). Liu predicted pressure-induced phase transformations at a few hundred kilobars for all the rare-earth elements. With the exceptions of Pm, Er, Tm, and Lu, all the rare-earth elements have been found to have one or more high-pressure polymorphs (*2–6*). The presence of a high-pressure phase of Tm was reported by Liu (*1*). Identification of the crystal structure of the high-pressure phase of Tm is reported here.

The changes in volume and electrical resistance of Tm with pressure up to 40 and 100 kbar, respectively, were investigated by Bridgman (*3*). The latter figure should be reduced to about 76 kbar after the revision of the pressure scale by Kennedy and LaMori (*7*). No irregularities in Tm were reported by Bridgman. Drickamer (*6*), however, observed some minor deviations from normal behavior in the resistivity of Tm at 60 to 80 and 150 to 160 kbar. Until now, this was the only study that suggested the transition in Tm. Several rare-earth elements have been studied by shock experiments (*8*), but Tm was not studied.

The sample of Tm used in this study is from an ingot with a stated purity of 99+ percent (*9*). The ingot was filed and the powdered sample was then compressed between the diamond anvils and examined by x-ray diffraction. The diamond-anvil press technique used for the high-pressure x-ray diffraction study has been described elsewhere (*10*).

Thulium crystallizes in the hexagonal close-packed (*hcp*) structure at 1 atm and room temperature, as many of the other rare-earth elements do. The lattice parameters of the sample used in this study at 1 atm and room temperature are $a_0 = 3.534 \pm 0.005$ Å and $c_0 = 5.573 \pm 0.008$ Å, which compare well with the values of $a_0 = 3.530$ Å and $c_0 = 5.575$ Å reported by Klemm and Bommer (*11*). The x-ray diffraction powder patterns of *hcp* metals at high pressure are complicated by preferred orientation effects, as reported by McWhan and Stevens (*5*). This effect is shown in Fig. 1b (run 3); the reflections with l greater than 1 are very weak or are not observable at high pressures.

To avoid interference in the diffraction patterns, no internal pressure calibrant was mixed with the sample. Hence, the pressure was estimated from the measured lattice parameters of Tm

Table 1. X-ray data for thulium at about 116 kbar and room temperature. Values I/I_{100} (obs), where I is intensity and I_{100} the intensity of the strongest line, were measured by a photoelectric densitometer for the stronger lines. The weak lines were estimated. The letter b denotes broad lines. Values of d(cal) for the *hcp* structure were computed from the assumed lattice parameters of $a = 3.327$ Å and $c = 5.247$ Å at 116 kbar (see Table 2). Values of d(cal) for the Sm-type structure were computed from $a = 3.327$ Å and $c = 23.48$ Å. The letter d denotes interplanar spacings, *hkl* are the indices of the crystal plane, and obs and cal stand for observed and calculated.

I/I_{100}(obs)	d(obs) (Å)	*hcp* structure		Sm-type structure	
		hkl	d(cal) (Å)	*hkl*	d(cal) (Å)
		100	2.881		
77	2.853			101	2.860
66b	2.793			012	2.798
100	2.576	101	2.526	009 104	2.609 2.586
73b	2.460			015	2.456
5	2.203			107	2.186
5	2.073			018	2.056
		102	1.940		
3	1.828			10.10	1.820
48	1.656	110	1.664	110	1.664
5	1.530			10.13	1.530
		103	1.495		
41	1.404	112	1.405	119 024	1.403 1.399
31b	1.385	201	1.389	205	1.377
		202	1.262		