The Chemistry of Reactive Lignin Intermediates. Part I. Transients in Coniferyl Alcohol Photolysis

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Photolysis of coniferyl alcohol produces a transient quinone methide. The rates of decay of this quinone methide have been measured in water, acetic acid, and propane-1.2-diol. The possible significance of the quinone methide in lignin biosynthesis is discussed.

Two classes of reactive intermediates have been implicated in lignin biosynthesis. The first of these, the phenoxyl radicals, have generally been considered [1] to form the basic lignin polymer by coupling reactions. The second, the quinone methides, are thought to be responsible for the formation of minor linkages in the lignin polymer and for the formation of lignin–carbohydrate bonds.[1]

The transient participation of phenoxyl radicals is consistent with the structures of the lignols isolated [1] from the enzymatic polymerizations of the lignin precursors, the p-coumaryl alcohols, and has also been inferred by the detection of a weak, unresolved e.s.r. signal [2] during the initial stages of these polymerizations. Similarly the isolation [1] of lignols which appear to have been formed by the addition of water or sucrose to a quinone methide, and the observation [3] of transient yellow colours during the enzymatic polymerizations have been attributed to quinone methide formation.

Further studies using enzymes to produce and more fully characterize the reactive intermediates of lignin biosynthesis are obviously desirable. Their usefulness in this respect is, however, limited by the low concentrations and short lifetimes of the intermediates which they produce. In a search for alternative methods of synthesis we decided to study the flash photolysis of coniferyl alcohol (Ia) and related compounds.

There were two reasons for anticipating that the flash photolysis of coniferyl alcohol would give rise to biosynthetically important intermediates, and in particular its phenoxyl radical. First, photolysis of coniferyl alcohol, like the enzymatic reaction, has been reported [4] to produce artificial lignin very similar to that isolated from sprucewood. Secondly, flash photolysis of phenols [5,6] in general gives rise to phenoxyl radicals.

EXPERIMENTAL

Coniferyl alcohol was synthesized [7] from vanillin. It was purified by column chromatography on silica gel and gave the expected u.v. and n.m.r. spectra.

The solvents used for the photolysis experiments were ethanol (AnalaR; B.D.H.), water (redistilled), propane-1,2-diol (May and Baker), diethyl ether (AnalaR; B.D.H.), acetic acid (AnalaR; B.D.H.), and carbon tetrachloride (AnalaR; B.D.H.). The blank solvents gave no detectable transients upon flash photolysis.

Samples were outgassed by a repetitive freeze-thaw process. U.v. absorption spectra were recorded on a Beckman DK2 spectrophotometer. The quantum yields were determined at 253·7 nm using a low pressure mercury arc (15 W) as source. Benzophenone (twice recrystallized from ethanol) in ethanol was used as an actinometer. The flash photolysis apparatus (Northern Precision Co.; type F.P. 1P) gave a flash duration of about 70 μs. In the flash experiments the coniferyl alcohol concentrations were 10^{-4}—10^{-6}M. Transient spectra were either determined from the oscilloscope traces at different monitoring wavelengths and corrected for the progressive effects of reactant disappearance and photoproduct formation, or, when their lifetime permitted, they were measured on a DK2 spectrophotometer.

The products of irradiation of coniferyl alcohol (350 mg) in acetic acid (200 ml) were obtained by photolysis with a low pressure mercury lamp in a 1-l Hanovia photochemical reactor for 2 h. They were isolated and purified by preparative t.l.c.

RESULTS

Flash photolysis of coniferyl alcohol in water, ethanol, propane-1,2-diol, acetic acid, carbon tetrachloride, or ether produced transients having absorption at $ca.$ 350 nm (Figure). The yield of transient was markedly higher for photolysis in acetic acid or water than for photolysis in the other solvents, and was $ca.$ 0 in ether. In all the solvents studied, the transient absorption grew in 0·3—1 ms; in addition, in carbon tetrachloride it appeared to be accompanied by a short-lived transient of half life $ca.$ 0·5 ms which absorbed in the 330—340 nm region.

The kinetics of disappearance of the major transient in water were first order $(k_1 = 2\cdot0 \pm 0\cdot3 \times 10^{-3}\text{ s}^{-1})$ and largely independent of the concentration of coniferyl alcohol over the range 10^{-4}—10^{-6}M. In acetic acid the rate of disappearance was also closely first order but was much greater $(k_1 = 0\cdot8 \pm 0\cdot15\text{ s}^{-1})$. On the other hand, the rate of disappearance of the transient in propane-1,2-diol was of second order $(k_2 = 2 \pm 0\cdot3 \times 10^{-2}\text{ }\varepsilon_{350}\text{ mol l}^{-1}\text{ s}^{-1})$ and in carbon tetrachloride or in ethanol it did not fit equations for first or second order reactions. In all solvents, except possibly carbon tetrachloride, it appeared to be independent of the presence or absence of atmospheric oxygen.

The quantum yield of disappearance of coniferyl alcohol

[1] J. M. Harkin in 'Oxidative Coupling of Phenols,' eds. W. I. Taylor and A. R. Battersby, Marcel Dekker, New York, 1967, pp. 263—300.

[2] K. Freudenberg, C.-L. Chen, J. M. Harkin, H. Nimz, and H. Renner, *Chem. Comm.*, 1965, 224.

[3] K. Freudenberg, G. Grion, and J. M. Harkin, *Angew. Chem.*, 1958, **70**, 743.

[4] K. Freudenberg and H. Dietrich, *Chem. Ber.*, 1953, **86**, 1157.

[5] E. J. Land, G. Porter, and E. Strachan, *Trans. Faraday Soc.*, 1961, **57**, 1885.

[6] E. J. Land and G. Porter, *Trans. Faraday Soc.*, 1963, **59**, 2016.

[7] D. Gagnaire, C. Lacoste, and D. Robert, *Bull. Soc. chim. France*, 1970, 1067.

was measured in diethyl ether, in ethanol, and in water. In the former solvent it was *ca.* 0; in the latter solvents it was initially *ca.* 0·2 and 0·4 respectively and appeared to decrease as the irradiation proceeded, reaching a minimum value of *ca.* 0·06 once the concentration of photoproducts was of the order of that of the residual coniferyl alcohol.

T.l.c. of the crude reaction mixture from the irradiation of coniferyl alcohol in acetic acid separated two major products, both of which had a greater R_F than coniferyl alcohol. The more polar product occurred in lower yield and was found, by comparison with an authentic sample, to be vanillin. The other was isolated as an oil in *ca.* 20% yield. Its u.v., i.r., and n.m.r. spectra indicated that it was coniferyl acetate (Ib). Thus its u.v. spectrum (Figure, line e) was characteristic of a *p*-hydroxy-styrene such as coniferyl alcohol, its major absorption band
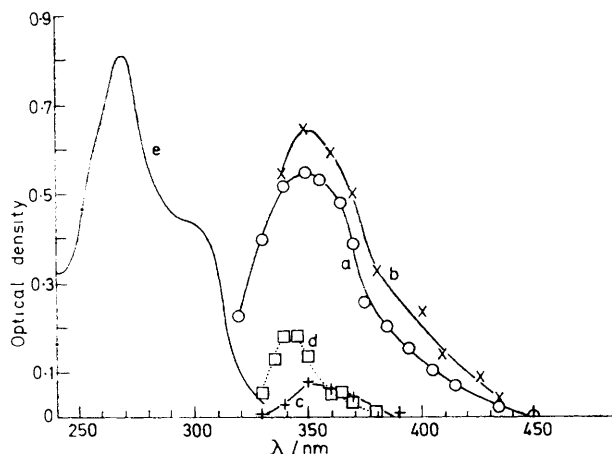
Transient absorption spectra obtained upon flash photolysis of coniferyl alcohol in a, water; b, acetic acid; c, ethanol; and d, carbon tetrachloride; delay time 0·1 s; e, absorption spectrum of coniferyl acetate (Ib) in ethanol

exhibiting a shift from 268 to 292 nm on addition of base. The i.r. spectrum showed a strong absorption at 1730 cm^{-1}, attributable to an aliphatic C=O stretching vibration, and the n.m.r. spectrum was that expected for coniferyl acetate (Ib), with δ 3·86 (3H, s, OMe) 2·08 (3H, s, OAc), and 6·90 p.p.m. (3H, ArH). The vinyl and methylene protons formed an ABX$_2$ system with coupling constants (J_{AX} 0, J_{BX} 5, and J_{AB} 16 Hz) equal to those of the corresponding protons in coniferyl alcohol. However, the X$_2$ doublet of the acetate at δ 4·69 p.p.m. had undergone the expected downfield shift from the position (δ 4·29 p.p.m.) of the methylene doublet in coniferyl alcohol.

DISCUSSION

The apparent unreactivity of the major transient towards atmospheric oxygen suggests that it is not the phenoxyl radical of coniferyl alcohol. Instead, it has the kinetic and spectral properties of the quinone methide (II), which has already been observed[8] as a transient during the peroxidase oxidation of eugenol. The slow unimolecular decay of the transient in water

and the markedly faster unimolecular decay in acetic acid are reactions typical of quinone methides, which are reported[9] to add hydroxy-compounds at rates which are apparently proportional to their dissociation constants. Thus the transient does not appear to react with dry propane-1,2-diol at all, but slowly disappears by a second-order process in this solvent, as do other quinone methides in unreactive solvents.

The addition of water to the quinone methide (II) would regenerate coniferyl alcohol. Most of the quinone methide formed in water appears to react in this way and this reaction must lower the quantum yield of disappearance observed in this solvent. At the same time some of the quinone methide probably reacts with coniferyl alcohol and phenolic photoproducts to produce di- and higher lignols and ultimately photo-lignin, which precipitates from the solution.

Addition of acetic acid to quinone methide (II) would give the same acetate (Ib) that was isolated from the photolysis of coniferyl alcohol in acetic acid. Formation of this acetate is thus strong evidence for the transient formation of the quinone methide (II).

Quinone methides have been previously[10] observed as transients formed by proton exchange during the photolysis of isoprenoid quinones. They have not been reported as transients during the photolysis of phenols, but their presence would not be unexpected in such reactions since, for example, certain substituted phenoxyl radicals are known[11] to disproportionate to quinone methides. It thus seems that there are at least two possible mechanisms by which photolysis of coniferyl alcohol could give rise to the quinone methide (II): (*a*) a polar mechanism involving consecutive or synchronous losses of the phenolic proton and the aliphatic hydroxy-group (Scheme) or (*b*) a reaction involving the phenoxyl radical of coniferyl alcohol.

SCHEME

The high yields of the quinone methide (II) formed upon photolysis of coniferyl alcohol in water or in acetic acid relative to those formed in less polar solvents like ethanol, propanediol, and carbon tetrachloride, and the almost zero yield of the quinone methide (II) upon
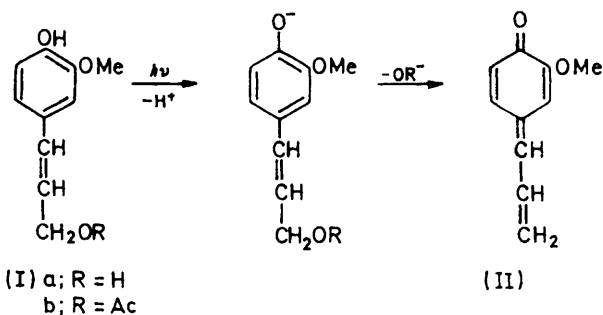
[8] J. C. Pew, W. J. Connors, and A. Kunishi, in 'Chimie et Biochimie de La Lignine, de la Cellulose et des Hemicelluloses. Actes du Symposium International de Grenoble, Juillet 1964,' Imprimeries Reunies de Chambery, 1965, pp. 229—245.

[9] K. Freudenberg in 'Constitution and Biosynthesis of Lignin,' K. Freudenberg and A. C. Neish, Springer Verlag, New York, 1968, p. 94.

[10] G. Leary, *J. Chem. Soc.* (*A*), 1971, 2248.

[11] C. D. Cook and B. E. Norcross, *J. Amer. Chem. Soc.*, 1956, **78**, 3797.

photolysis in ether, strongly suggests that the quinone methide (II) is most readily formed from coniferyl alcohol by a polar mechanism as in the Scheme. The pK_a value of excited coniferyl alcohol is almost certainly sufficiently low for the molecule to dissociate in polar solvents. The mesomeric anion thus formed could then react by loss of hydroxide ion to give the transient (II). If this mechanism is correct, the measurable growth of the quinone methide (II) in 0·3—1 ms following the exciting flash could suggest that the anion has a measurable lifetime in dilute solution.

At the same time, the formation of the quinone methide (II) upon photolysis of coniferyl alcohol in carbon tetrachloride is evidence for the separate existence of a radical mechanism. The accompanying short-lived transient observed in this solvent might well be the phenoxyl radical of coniferyl alcohol. If so its half life, *ca.* 0·5 ms, is of the order of those of other unhindered phenoxyl radicals and is considerably less than the 45 s half life attributed [2] to this species in aqueous dioxan.

This study of the photolysis of coniferyl alcohol was in part stimulated by the report [12] that soluble native spruce lignin and photo-lignin derived from coniferyl alcohol in water are chemically indistinguishable. Since spruce lignin is equally claimed [1] to be duplicated by a dehydrogenation polymer (DHP) lignin produced by the enzymatic polymerization of a specific mixture of coumaryl alcohols, it would appear from product analysis that DHP and photo-lignin should also be identical.

There seems little doubt that DHP lignin is primarily formed by the polymerization of free radicals. The present finding, that a quinone methide, not a free radical, is the transient detected when coniferyl alcohol is photolysed in water, suggests that photo-lignin is formed by addition of phenols to quinone methides. Thus, contrary to the above claims, it seems that DHP and photo-lignin must be different types of polymer. It remains to be shown which of these lignins (if either) is truly characteristic of natural lignin and hence whether, as is currently accepted, free radicals are more important than quinone methides as intermediates in lignin biosynthesis. In this connection, it is noted that quinone methides like that detected here can be produced by non-photolytic, biochemical means.[8]

[12] F. E. Brauns and D. A. Brauns, ' The Chemistry of Lignin,' supp. vol., Academic Press, New York, 1960, p. 702.