[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, YALE UNIVERSITY]

Reactions of Phenylpropene Derivatives with Nitrous Acid[1]

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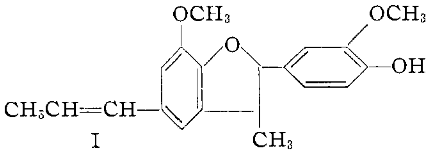
Several reactions of *p*-hydroxyphenylpropene derivatives with nitrous acid have been studied. Dehydrodiisoeugenol and a polymeric compound were obtained from isoeugenol, and a dehydropolymer was obtained from *p*-coumaryl alcohol. When treated with nitrous acid, *p*-hydroxychalcones yield nitro derivatives. The possible relation of the oxidative action of nitrous acid on *p*-hydroxyphenylpropenes to the formation of lignin precursors is discussed.

The finding that *p*-hydroxycinnamic acid (*p*-coumaric acid) is oxidized readily by nitrous acid at acid *p*H values,[3] and the widespread occurrence, in plant tissues, of nitrate reductase, led to the suggestion[3] that the nitrite generated by the enzymic reduction of nitrate may participate in the oxidation of phenylpropene derivatives currently believed to be precursors of the natural lignins.[4,5] In attempts to approach the problem of lignin biosynthesis, previous investigators have examined the action of mild oxidants ($FeCl_3$, O_2, H_2O_2) on selected phenylpropene derivatives (isoeugenol, coniferyl alcohol, ferulic acid, eugenol, etc.). Of special interest are the studies of Erdtman[6] and of Freudenberg[7] who showed that the oxidation of isoeugenol by $FeCl_3$ leads to the formation of the dimeric compound dehydrodiisoeugenol (I), whose structure 2-(4′-hydroxy-3-methoxyphenyl)-3-methyl-5-propenyl-7-methoxycoumaran was established by degradation studies.



I

An analogous dimeric coumaran derivative was isolated[8] after the action of O_2 on coniferyl alcohol at *p*H values near 6, in the presence of an enzyme preparation from mushrooms. Such dimeric compounds have therefore been considered to represent intermediates in the polymerization of oxidized phenylpropene derivatives to the polymeric lignins.[9]

In view of these results, and their possible importance for the problem of lignin biosynthesis, it seemed of interest to examine the action of nitrite on isoeugenol and related phenylpropene derivatives. It was found that, at *p*H 6, nitrite readily effects the conversion of isoeugenol to dehydrodiisoeugenol; a yield of 35–40% of the theory was obtained under the conditions described in the Experimental section. The identity of this product with that described by Erdtman[6] was established by the analytical data, and by the melting points of the product, of the *p*-nitrobenzoyl ester, and of the monomethyl ether. In addition, a less well-defined product (softens at 90–110°) was obtained from the reaction mixture; a molecular weight determination of this material suggested that it may be composed, on the average, of six C_9-units joined together. Like soluble lignins, the polymer gave a dark red color with the phloroglucinol–HCl reagent. The possibility exists, therefore, that the material contains lignin-like substances formed by the polymerization of oxidized C_9-units.

It will be noted from Fig. 1 that the shift in the absorption maxima for isoeugenol (258 mμ, molar absorbance 14,800), dehydrodiisoeugenol (273 mμ, molar absorbance 18,200) and the 90–110° product (282 mμ), also is in accord with the view that the two latter compounds represent different stages in the polymerization of oxidized isoeugenol. Calculation of the molar absorbance per C_9-unit (assumed molecular weight 108) at their respective maxima gives the following values: isoeugenol, 9,500; dehydrodiisoeugenol, 6,100; the 90–110° product, 2,380.

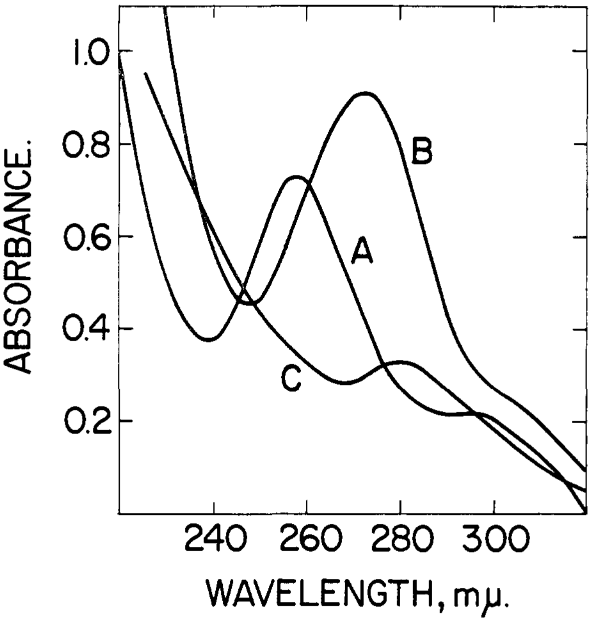


Fig. 1.—Absorption spectra of isoeugenol, 5×10^{-5} *M* (curve A); of dehydrodiisoeugenol, 5×10^{-5} *M* (curve B); and of polymer (15.1 mg. per liter) formed by the action of nitrite on isoeugenol (curve C); solvent, 95% ethanol, 1-cm. cells.

Treatment of *p*-coumaryl alcohol with nitrite (2 moles of nitrite per mole of alcohol) at *p*H 6 gave a water-insoluble product whose absorption spec-

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[2] Postdoctoral Fellow of the Greek Fellowship Foundation.

[3] C. Zioudrou, W. L. Meyer and J. S. Fruton, THIS JOURNAL, **79**, 4114 (1957).

[4] K. Freudenberg, *Angew. Chem.*, **68**, 84, 508 (1956).

[5] S. M. Siegel, *Quant. Rev. Biol.*, **31**, 1 (1956).

[6] H. Erdtman, *Biochem. Z.*, **258**, 172 (1933); *Ann. Chem.*, **503**, 283 (1933).

[7] K. Freudenberg and H. Richtzenhain, *ibid.*, **552**, 126 (1942).

[8] K. Freudenberg and H. H. Hübner, *Ber.*, **85**, 1181 (1952).

[9] K. Freudenberg, *Fortsch. Chem. org. Naturstoffe*, **11**, 43 (1954).

trum resembled that reported[10] for the polymer obtained by treatment of *p*-coumaryl alcohol with a mushroom extract at *p*H 7. The molar absorbance of *p*-coumaryl alcohol at 260 mμ is 20,000 (Fig. 2); when calculated per C_9-unit, its molar absorbance is 14,000, and that of the polymer formed in the presence of nitrite is 5,100 at 265 mμ (Fig. 2). In addition to the decrease in absorbance per C_9-unit, comparison of two spectra shows an almost complete disappearance, in that of the polymer, of the characteristic absorption band at 295 mμ of the ethylenic double bond. A control experiment at *p*H 6, in the absence of nitrite, gave no evidence of the formation of polymers; at acid *p*H values (*ca.* *p*H 3), coumaryl alcohol undergoes polymerization.[10]

Reaction of Chalcones with Nitrous Acid.—In view of the structural similarity between derivatives of *p*-coumaric acid and those of benzalacetophenone (chalcone), it was of interest to examine the reaction of the latter compounds with HNO_2. Previous work had shown[3] that whereas *p*-coumaric acid is subjected to oxidative decarboxylation, the major product derived from the methyl ester of *p*-coumaric acid is the 3-nitro derivative. More recent work has demonstrated that this nitration depends on the presence of the phenolic hydroxyl group, since *p*-methoxycinnamic acid methyl ester does not undergo any observable change under comparable experimental conditions. *p*-Methoxycinnamic acid appears to be converted by HNO_2 to products that exhibit maximum absorption at longer wave lengths; however, no CO_2 evolution was observed, and attempts to isolate a distinctive product were not successful.

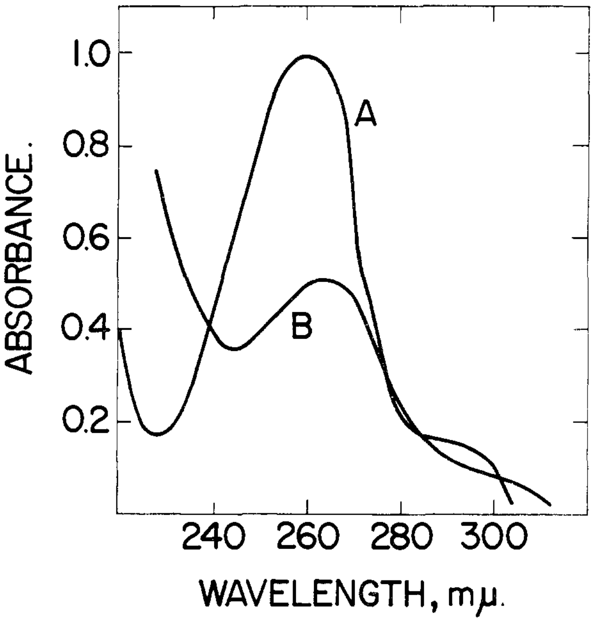

Fig. 2.—Absorption spectra of *p*-coumaryl alcohol, 5×10^{-5} *M* (curve A), and of polymer (10.8 mg. per liter) formed by the action of nitrite (curve B); solvent, 95% ethanol, 1-cm. cells.

As with the methyl ester of *p*-coumaric acid, the principal product of the reaction of 4,4′-dihydroxychalcone with nitrous acid was 3′-nitro-4,4′-dihydroxychalcone. The identity of this product was established by alkaline degradation to *p*-hydroxyacetophenone and 3-nitro-4-hydroxybenzoic acid. The possibility that the product was 4,4′-dihydroxy-3-oximinoflavanone[11] was excluded by comparison of the dimethoxy derivative of the product with an authentic sample of 4,4′-dimethoxy-3-oximinoflavonone.[12] Similarly, with 2,4′-dihydroxychalcone, treatment with nitrous acid gave, as the principal product, 3′-nitro-2,4′-dihydroxychalcone.

Experimental

Reaction of Isoeugenol with Nitrite.—To a solution of $NaNO_2$ (8.3 g., 0.13 mole) in one liter of 0.06 *M* phosphate buffer (*p*H 6.0) was added, with stirring, a solution of isoeugenol (10 g., 0.06 mole) in 300 ml. of acetone. The mixture became yellow and turbid after 5 min., and crystals separated from the solution after it had stood at room temperature for 6 hours. After 30 hours, the precipitate (3.8 g.) was collected; after recrystallization from methanol it melted at 132–133° (reported[6] 133–134°). Calcd. for $C_{20}H_{22}O_4$ (326.4): C, 73.5; H, 6.7. Found: C, 73.2; H, 6.8; mol. wt. (Rast method), 329. Treatment with *p*-nitrobenzoyl chloride gave the mono-*p*-nitrobenzoyl ester, m.p. 154° (reported[6] 156–157°). Calcd. for $C_{27}H_{25}NO_7$ (475.5): N, 2.98. Found: N, 2.94. Treatment with dimethyl sulfate gave the monomethyl ether, m.p. 125–126° (reported[6] 126°). Upon treatment with concd. H_2SO_4, the dimer gives a blood-red color, which is changed to blue-green when water is added. The compound gives a light pink color with the phloroglucinol–HCl reagent. It is soluble in hot methanol, hot ethanol, chloroform, ethyl acetate and acetone. The absorption spectra of isoeugenol and dehydrodiisoeugenol are shown in Fig. 1. The infrared spectrum (KBr press disk) of dehydroisoeugenol showed the following maxima (in cm.$^{-1}$): 3400(s), 2850(w), 1610(m), 1545(s), 1520(s), 1470–1450(m), 1380(m), 1280(s), 1230–1210(m), 1160–1110(m), 1030(s), 960(w), 880(w), 855(w).

The filtrate obtained by the removal of the crystalline dehydrodiisoeugenol was concentrated to about 800 ml. and chilled; a yellow solid (2 g.) separated. This product is extremely soluble in methanol and is sparingly soluble in water; it softens at 90–110° dec. A determination of the apparent molecular weight by the Rast method gave a value of 985, suggesting that it is composed, on the average, of six C_9-units. The product was N-free (sodium fusion, Kjeldahl analysis). On treatment with phloroglucinol–HCl, the product gave a dark red color. The absorption spectrum of this material is given in Fig. 1.

The filtrate obtained by the removal of the product described in the preceding paragraph was concentrated to dryness under reduced pressure, and extracted with 30 ml. of methanol. From the methanolic extract, a brown powder (0.5 g.) was obtained, which gave a dark-red color with phloroglucinol–HCl.

When a mixture of 100 ml. of 0.06 *M* phosphate buffer (*p*H 6.0) and a solution of 1 g. of isoeugenol in 30 ml. of acetone was kept at room temperature for 2 days, no crystallization was noted.

Reaction of *p*-Hydroxycinnamic Derivatives with HNO_2.—To a solution of 110 mg. (0.57 millimole) of *p*-methoxycinnamic acid methyl ester[13] (m.p. 88–89°) in 50 ml. of dioxane–water (1:1 by volume), 85 mg. (1.2 millimoles) of $NaNO_2$ was added, and the solution was acidified to *p*H 2 with HCl. Spectrophotometric observation of the course of the reaction showed no change in the ultraviolet absorption spectrum over a 2-hr. period. When 0.24 g. (1.3 millimoles) of *p*-methoxycinnamic acid[14] in 100 ml. of dioxane–water was treated with 0.18 g. of $NaNO_2$ (2.6 millimoles), and the solution was acidified to *p*H 2, after one hour the absorption maximum at 300 mμ shifted to 310 mμ. without appreciable

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(10) K. Freudenberg and G. Gehrke, *Ber.*, **84**, 443 (1951).

(11) By analogy to the suggestion that an oximino compound is an intermediate in the reaction of N-acetyldehydrotyrosine with nitrous acid [G. Taborsky, P. S. Cammarata and J. S. Fruton, *J. Biol. Chem.*, **226**, 103 (1957)].

(12) S. v. Kostanecki and M. Widner, *Ber.*, **37**, 4161 (1904).

(13) W. Will, *ibid.*, **20**, 294 (1887).

(14) T. Rotarski, *ibid.*, **41**, 1996 (1908).

change in absorbance. No CO_2 evolution was observed, and attempts to isolate a crystalline product were unsuccessful.

Reaction of 4,4'-Dihydroxychalcone with HNO_2.—To a solution of 4,4'-dihydroxychalcone[15] (2 g., 0.0083 mole) in dioxane (60 ml.) was added a solution of $NaNO_2$ (1.2 g., 0.0174 mole) in 60 ml. of water. The mixture was acidified with 5 N HCl to *p*H 2 and shaken for 3 hours at room temperature, and the crystalline precipitate that separated was filtered, washed with water and ethanol, and dried; m.p. 287–290°. Additional material (m.p. 285–290°) crystallized from the filtrate when it was allowed to stand at room temperature for 8 hours. The orange-red product (total yield 1.25 g.) was recrystallized twice from ethyl acetate-benzene; m.p. 290°. Calcd. for $C_{15}H_{11}NO_5$ (285.3): C, 63.2; H, 3.9; N, 4.9. Found: C, 63.0; H, 4.1; N, 4.7.

On acetylation of 0.1 g. of the product with 4 ml. of acetic anhydride in the presence of 0.25 g. of sodium acetate, a crystalline product (0.1 g., m.p. 160°) was obtained. Calcd. for $C_{19}H_{15}NO_7$ (369.3): C, 61.7; H, 4.1; N, 3.8. Found: C, 62.0; H, 4.1; N, 3.7.

On methylation of 0.2 g. of the product with 0.4 ml. of dimethyl sulfate in the presence of methanolic KOH, the dimethoxy derivative was obtained. After recrystallization from methanol, it melted at 167° dec. Calcd. for $C_{17}H_{15}NO_5$ (313.3): N, 4.5. Found: N, 4.5. A mixed m.p. determination with an authentic sample of 4,4'-dimethoxy-3-oximinoflavanone[11] (m.p. 168–170° dec.) gave a melting point of 140–143°, showing that the two compounds are not identical.

Alkaline degradation of the product (1 g.) with 50% KOH (25 ml.) at 170–180° for 3 hours, followed by neutralization with CO_2, and extraction with ether, gave, upon evaporation of the ethereal solution, *p*-hydroxyacetophenone (m.p. 105°, no depression of mixed m.p. with an authentic sample). The neutralized aqueous solution was acidified and extracted with ether; evaporation of the ethereal extract gave 3-nitro-4-hydroxybenzoic acid (m.p. 183°, reported[16] 183°). Calculated for $C_7H_5NO_5$ (183.1): C, 45.9; H, 2.7; N, 7.6. Found: C, 46.1; H, 2.7; N, 7.4. The structure of the product obtained by treatment of 4,4'-dihydroxychalcone with HNO_2 is therefore 3'-nitro-4,4'-dihydroxychalcone. The dimethoxy derivative of this compound has been reported to melt at 160° dec.[17]

Reaction of 2,4'-Dihydroxychalcone with HNO_2.—This chalcone[14] was treated with HNO_2 in the same manner as the 4,4'-isomer to yield 1.8 g. of a product which, after recrystallization from ethanol, melted at 230°. Calcd. for $C_{15}H_{11}NO_5$ (285.3): C, 63.2; H, 3.9; N, 4.9. Found: C, 62.9; H, 4.1; N, 4.8. On acetylation, the diacetate (m.p. 93–94°) was obtained. Calcd. for $C_{19}H_{15}NO_7$ (369.3): N, 3.7. Found: N, 3.8. On degradation with alkali as above, followed by acidification and steam distillation, *o*-hydroxyacetophenone was identified by its ultraviolet absorption spectrum (as compared with that of an authentic sample); after extraction of the residue with ether, 3-nitro-4-hydroxybenzoic acid (m.p. 183°) was isolated, indicating that the product of the reaction with HNO_2 is 3'-nitro-2,4'-dihydroxychalcone.

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[15] T. A. Geissman and R. O. Clinton, This Journal, **68**, 697 (1946).

[16] P. Griess, *Ber.*, **20**, 408 (1887).

[17] P. Pfeiffer and B. Segall, *Ann. Chem.*, **460**, 130 (1928).

New Haven, Conn.

[Contribution from the Research Department, Standard Oil Co. (Indiana)]

Disproportionation of Alkylbenzenes. IV. Ethylbenzene and Diethylbenzene

By D. A. McCaulay and A. P. Lien

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In the presence of hydrogen fluoride plus excess boron trifluoride, ethylbenzene disproportionates rapidly and soon reaches a primary equilibrium having the composition: benzene, 45%; ethylbenzene, 10%; *m*-diethylbenzene, 45%. Little triethylbenzene is formed because the *m*-diethylbenzene takes on a proton and is tied up as a positively charged complex. However, at higher temperatures or after longer times diethylbenzene gradually reacts and a new equilibrium is slowly approached containing four components: benzene, ethylbenzene, 1,3-diethylbenzene and 1,3,5-triethylbenzene. The equilibrium composition is found to be a function of the number of ethyl groups per benzene ring in the starting hydrocarbon mixture.

Ethylbenzene, in the presence of hydrogen fluoride plus excess boron trifluoride, disproportionates rapidly and almost completely at room temperature into benzene and *m*-diethylbenzene.[1] The completeness of reaction and the production of the *meta* isomer were attributed to the preferential formation of a complex of *m*-diethylbenzene with $HF–BF_3$. Although diethylbenzene as well as ethylbenzene should accept alkyl groups, no triethylbenzene was formed. This result was surmised to be a rate rather than an equilibrium effect. As part of a continued study of the reaction, therefore, ethylbenzene and diethylbenzene were disproportionated with longer reaction times and at higher temperatures. Under these more vigorous reaction conditions the product distribution was found to be different and to be a function of the ratio of ethyl groups to benzene rings present.

Experimental

Ethylbenzene, diethylbenzenes and triethylbenzenes from Eastman Kodak Co. were used without further purification. Commercial grades of hydrogen fluoride, 99.6% pure, and boron trifluoride, about 99% pure, were obtained from the Harshaw Chemical Co.

The experiments were carried out in a 1000-ml. Hastelloy autoclave fitted with a 1725-r.p.m. stirrer. Hydrocarbon, hydrogen fluoride and boron trifluoride were charged to the autoclave and the mixture was stirred at a controlled temperature. After reaction, the entire contents were withdrawn into crushed ice. The hydrocarbon product was separated into successive carbon-number fractions by distillation through a column of thirty theoretical plates. Individual fractions were identified by their physical properties and by spectrometric analysis.

Discussion

The results obtained on treating ethylbenzene with hydrogen fluoride plus a large excess of BF_3 are given in Table I. At low temperatures and short reaction times the products are benzene and *m*-diethylbenzene. At higher temperatures and at longer times, triethylbenzene appears and approaches 25 mole % of the mixture. The explanation for this behavior is that the first step reaches equilibrium rapidly; because a large excess of BF_3 is used, most of the primary product, *m*-diethylbenzene, is tied up as a positively charged aronium ion.

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[1] D. A. McCaulay and A. P. Lien, This Journal, **75**, 2407 (1953); (III) **76**, 2354 (1954); (V) **79**, 5808 (1957).