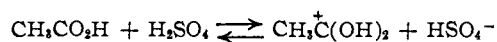


may contribute a degree of "disordering," *i.e.*, an entropy term $+Y$. The entropy of activation in the latter solvent then becomes $-X + Y$, which is less negative than $-X$. (ΔS° for the hexanoic acid: in water, $\hat{=}$ -16 , in acetic acid, $\hat{=}$ -33 , and similarly for the heptanoic acid.)

When small amounts of water are added, the solvent system obviously becomes very complicated; the water may promote ionization of the sulfuric acid, which is otherwise very incomplete²⁷



Also, owing to the low polarity of the acetic acid, water molecules or oxonium ions may become partially segregated in the neighborhood of the keto-acid molecules; in that case the environment of the

(27) H. A. E. Mackenzie and E. R. S. Winter, *Trans. Faraday Soc.*, **44**, 159 (1948).

reactant will be unlike that in anhydrous acetic acid and may tend toward conditions prevailing in aqueous solutions. This would account for the reaction parameters in "wet" acetic acid having values between those observed in the anhydrous solvent and those in aqueous solution. Inhibition of the ring-closure by acetic anhydride is believed to be due to the presence of acetylium ion, $\text{CH}_3\overset{+}{\text{C}}\text{O}$,²⁸ the latter must function as a strong acid and electrophilic reagent and may attack the basic centers of the keto-acid or the enolic double bond.

Acknowledgments.—The authors wish to thank Imperial Chemical Industries, Ltd., for a grant toward the cost of materials and Mr. P. R. Wood for some of the elementary analyses.

(28) H. Burton and P. F. C. Praill, *Quart. Revs.*, **6**, 314 (1952).

LONDON E.C. 3, ENGLAND

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, YALE UNIVERSITY]

Reactions of Nitrous Acid with *p*-Hydroxycinnamic Acid and its Derivatives¹

BY CHRISTINE ZILOUDROU,² WILLIAM L. MEYER AND JOSEPH S. FRUTON

RECEIVED MARCH 21, 1957

p-Hydroxycinnamic acid reacts with HNO_2 in dilute aqueous solution to form *p*-hydroxymandelaldehyde. With derivatives of *p*-hydroxycinnamic acid in which the carboxyl group is substituted (*e.g.*, the methyl ester), the predominant reaction is a nitration *ortho* to the phenolic hydroxyl. Because of the production of nitrite by the nitrate reductase of plants, the possibility exists that reactions of HNO_2 with *p*-hydroxycinnamic acid and its derivatives may occur in plant tissues.

Previous studies in this Laboratory^{3,4} have led to the finding that *N*-acetyldehydrotyrosine (*N*-acetyl-*p*-hydroxycinnamic acid) is readily converted by dilute HNO_2 to *N*-acetyl-*p*-hydroxymandelic acid amide. This conversion was first observed³ upon acidification of suspensions of *Escherichia coli* grown in a culture medium supplemented with acetyldehydrotyrosine. Subsequent studies⁴ showed that the role of the bacteria is to reduce nitrate (a component of the basal medium⁵) to nitrite, presumably by the action of nitrate reductase⁶; upon acidification of the culture filtrate to *pH* 4 or less, a rapid non-enzymic reaction between HNO_2 and acetyldehydrotyrosine ensues. Because of the widespread occurrence, among plants, of nitrate reductase and of *p*-hydroxycinnamic acid (*p*-coumaric acid) and its derivatives (*e.g.*, caffeic acid, coniferyl alcohol), the new reaction merited further study in relation to several problems of plant metabolism.

Reaction of *p*-Hydroxycinnamic Acid with HNO_2 .

—In analogy with the action of HNO_2 on acetyldehydrotyrosine, the expected product of the reaction with *p*-hydroxycinnamic acid is *p*-hydroxy-

mandelaldehyde, which does not appear to have been described previously; the isomeric 1,4'-dihydroxyacetophenone was prepared by Robinson.⁷ Upon treatment of *p*-hydroxycinnamic acid with 2 equivalents of HNO_2 at *pH* 2, CO_2 is evolved with the formation of a product whose strong reducing properties, absorption spectrum, elementary analysis, and derivatives (2,4-dinitrophenylhydrazones, semicarbazones, phenylosazones) support its identification as *p*-hydroxymandelaldehyde. On treatment with 2.5 moles of NaIO_4 per mole of product, the expected amount of formic acid was released, and *p*-hydroxybenzaldehyde (identified as its phenylhydrazone) was formed. However, with a 1:1 molar ratio of periodate to product, 50% of the expected formic acid was released more rapidly than the rest, suggesting that the *p*-hydroxymandelaldehyde is present in aqueous solution as a dimer that is slowly cleaved during the reaction with periodate. This possibility is supported by the fact that the semicarbazone isolated from aqueous solution gave an elementary analysis in agreement with the values calculated for a derivative of a dimer.

The rate of the reaction of *p*-hydroxycinnamic acid with nitrite is markedly dependent on *pH*. At 30°, in the presence of 0.11 *M* citric acid-sodium citrate buffer containing 4×10^{-3} *M* nitrite (introduced as sodium nitrite) the apparent first-order rate constants (in min.^{-1}) for the disappearance of *p*-hydroxycinnamic acid (initial concentration, 4×10^{-4} *M*) were found to be: *pH* 5.1, 0.0010; *pH* 4.1, 0.0068; *pH* 2.9, 0.092. At lower *pH*

(1) This work was supported by grants from the Rockefeller Foundation and from the National Science Foundation.

(2) Postdoctoral Fellow of the Greek Fellowship Foundation.

(3) J. S. Fruton, S. Simmonds and V. A. Smith, *J. Biol. Chem.*, **169**, 267 (1947).

(4) G. Taborsky, P. S. Cammarata and J. S. Fruton, *ibid.*, **226**, 103 (1957).

(5) C. H. Gray and E. L. Tatum, *Proc. Natl. Acad. Sci.*, **30**, 404 (1944).

(6) A. Nason, in W. D. McElroy and B. Glass, "Inorganic Nitrogen Metabolism," Johns Hopkins Press, Baltimore, Md., 1956, p. 109.

(7) R. Robinson, *J. Chem. Soc.*, 1464 (1928).

values, the rate was too rapid to be measured accurately with the available equipment. These rate constants were determined by observation of the decrease in the absorbance at the wave length corresponding to the maximum absorption of *p*-hydroxycinnamic acid; these maxima were (molar absorbance values being given in parentheses): pH 5.1, 286 m μ (19,600); pH 4.1, 305 m μ (19,200), pH 2.9, 308 m μ (21,600). The absorption spectrum of *p*-hydroxymandelaldehyde at acidic pH values has a maximum at 273 m μ (molar absorbance, 2620); near 300 m μ , the molar absorbance is about 950 (cf. Fig. 1). Beer's law was assumed to hold under the conditions of these experiments.

The effect of pH on the rate of the disappearance of *p*-hydroxycinnamic acid indicates that the reactive species is HNO₂ (pK' ca. 3.4) or a substance derived from it in acid solution. Although insufficient kinetic data are available for their use in the formulation of a mechanism of the reaction, the results are consistent with the hypothesis advanced previously⁴ that the reaction involves an attack by the nitrosonium ion (NO⁺), which may arise from nitrous acid by the reaction $\text{HNO}_2 + \text{H}^+ \rightarrow \text{NO}^+ + \text{H}_2\text{O}$.⁸

Reaction of Caffeic Acid with HNO₂.—When caffeic acid (3,4-dihydroxycinnamic acid) was treated with 2 equivalents of nitrous acid at pH 2, CO₂ was evolved, and an orange-colored solution resulted, from which no well-defined product could be isolated. The conversion of the initial product (possibly 3,4-dihydroxymandelaldehyde) to other substances is indicated by spectrophotometric observations. In acid solution, caffeic acid exhibits maximal absorption at 315 m μ (molar absorbance, 21,500). Two minutes after treatment with HNO₂ at room temperature, the absorption spectrum of the reaction mixture had changed to one exhibiting maximal absorption at 347 m μ , with a lesser peak at 284 m μ . During the succeeding 2 hours, the peak at 315 m μ gradually diminished, but the other peak was retained. From the reaction mixture (after 2 hours), there was obtained a brown amorphous material that is soluble in water, acetone and alcohol; it is insoluble in hydrochloric acid but dissolves in NaOH to give a dark red solution.

Nitration of *p*-Hydroxycinnamic Acid Derivatives by HNO₂.—It has long been known that phenols can be nitrated by means of nitrous acid (e.g., salicylic acid is converted to 5-nitrosalicylic acid). Presumably, the initial attack by NO⁺ at the position *ortho* to the phenolic hydroxyl gives rise to the nitroso compound, which is oxidized to the nitro derivative.⁹ It is of interest therefore that, in the reaction of *p*-hydroxycinnamic acid or of its α -acetamido derivative with HNO₂ under the conditions described above, no evidence was obtained of nitration. This suggests that the α -carbon of *p*-hydroxycinnamic acid is more reactive toward an electrophilic reagent such as NO⁺ than is the carbon *ortho* to the phenolic hydroxyl group. However, when the methyl ester of *p*-hydroxycinnamic

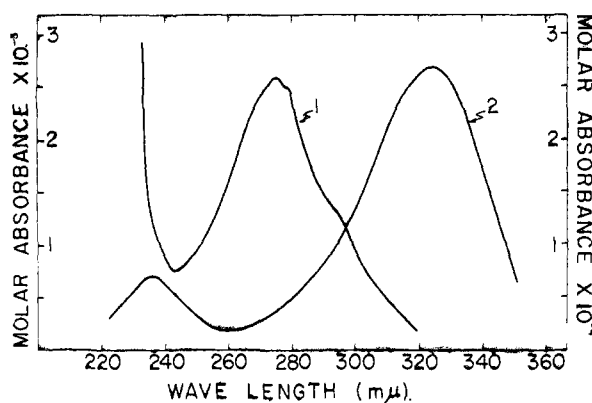


Fig. 1.—Absorption spectra of *p*-hydroxymandelaldehyde at pH 2 (curve 1, ordinate at left) and at pH 10 (curve 2, ordinate at right).

acid was treated with one equivalent of HNO₂ in dioxane-water (pH ca. 2), the principal product was 3-nitro-4-hydroxycinnamic acid methyl ester, indicating a preferential attack of the ring by the nitrosonium ion. In this case, decarboxylation cannot occur, as with *p*-hydroxycinnamic acid, thus removing from the over-all process a strongly exergonic reaction that may be expected to favor nitrosation of the α -carbon. A similar situation appears to apply in the action of HNO₂ on derivatives of benzalacetophenone such as 4,4'-dihydroxychalcone; from the reaction mixture in dioxane-water a product was obtained which, on alkaline degradation, gave 3-nitro-4-hydroxybenzoic acid. Studies on the action of nitrous acid on hydroxychalcones will be reported in a subsequent communication from this Laboratory.

Discussion.—The extensive studies of Freudenberg¹⁰ and others have drawn renewed attention to the possibility that the biosynthesis of lignin involves the oxidation and polymerization of phenylpropane derivatives. This view is supported by isotope experiments indicating the utilization of *p*-hydroxyphenylpyruvic acid for lignin formation.¹¹ Although speculation about the role of nitrite in lignin formation would be premature at present, the reactions described in the present communication, when considered in relation to the widespread occurrence of nitrate reductase in plants, suggest that consideration be given to the possibility that *p*-hydroxycinnamic acid derivatives may be oxidized in plant tissues, not only in the H₂O₂-dependent reactions already examined,¹² but also in reactions that involve HNO₂ as the oxidant.

Experimental

Formation of *p*-Hydroxymandelaldehyde.—To a solution of *p*-hydroxycinnamic acid (3 g., 0.0182 mole) in 50 ml. of freshly distilled dioxane was added a solution of sodium nitrite (2.45 g., 0.036 mole) in 50 ml. of water; the mixture was adjusted to pH 2 by the addition of about 4 ml. of 5 *N* hydrochloric acid. The resulting yellow solution was shaken for 1.5 hours at room temperature, and then was concentrated to dryness under reduced pressure at 40°. The residue was extracted 8 times with 50-ml. portions of hot acetone, and the combined acetone extracts were concen-

(8) F. Seel and R. S. Schwachel, *Z. anorg. allgem. Chem.*, **274**, 169 (1954).

(9) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 285.

(10) K. Freudenberg, *Angew. Chem.*, **68**, 54, 508 (1956).

(11) W. J. Schubert, S. N. Acerbi and F. F. Nord, *THIS JOURNAL*, **79**, 251 (1957).

(12) S. M. Siegel, *Quart. Rev. Biol.*, **31**, 1 (1956).

trated to dryness under reduced pressure at room temperature. The residue was treated with 80 ml. of ice-cold water, and the insoluble material was filtered and washed with 20 ml. of cold acetone. The dry product (0.9 g.) melted at 181–183° dec. After two recrystallizations from glacial acetic acid, it melted at 183–185° dec. Calcd. for $C_{14}H_{13}O_4$ (152.1): C, 63.1; H, 5.3. Found: C, 62.5; H, 5.4. A test for nitrogen was negative. The substance is soluble in hot acetone and glacial acetic acid, and is sparingly soluble in hot ethanol and in water. It reduces Fehling solution. Its absorption spectra at pH 2 and 10 are given in Fig. 1.

A 2,4-dinitrophenylhydrazone was prepared by the addition of 5 ml. of the 2,4-dinitrophenylhydrazine reagent¹³ to a solution of 0.1 g. of the product in 20 ml. of ethanol. The red crystalline powder (0.12 g.) melted at 277–280°; after two recrystallizations from ethanol–ethyl acetate, it melted at 280° dec. Calcd. for $C_{14}H_{13}N_4O_8$ (332.3): C, 50.6; H, 3.6; N, 16.9. Found: C, 50.7; H, 3.8; N, 17.1.

A semicarbazone was prepared by the addition of 0.25 g. of semicarbazide hydrochloride to a hot solution of 0.2 g. of the product in 10 ml. of water containing 0.6 g. of sodium acetate. The mixture was heated for 5 minutes on the steam-bath and chilled; the resulting crystalline product (0.2 g.) melted at 165° dec. After 3 recrystallizations from water it melted at 168° dec. Calcd. for a semicarbazone of a dimer, $C_{17}H_{15}N_3O_8$ (361.35): C, 56.5; H, 5.2; N, 11.6. Found: C, 56.4; H, 5.4; N, 11.6.

An osone was prepared by the addition of 0.6 g. of phenylhydrazine hydrochloride and of 0.5 ml. of saturated sodium bisulfite solution (to prevent the formation of tarry products) to a hot solution of 0.2 g. of the product in 10 ml. of water containing 1.1 g. of sodium acetate. The mixture was heated for about 20 minutes on the steam-bath and chilled; the resulting yellow product (0.25 g.) melted at 190°. After 5 recrystallizations from ethanol–water it melted at 198–199°. Calcd. for $C_{20}H_{13}N_4O$ (330.3): C, 72.7; H, 5.45; N, 16.9. Found: C, 72.3; H, 5.6; N, 16.7.

For the oxidation of the product with periodate, 600 mg. (2.6 millimoles) of $NaIO_4$ was added to a solution of 200 mg. (1.3 millimoles) of *p*-hydroxymandelaldehyde in 300

ml. of water. The mixture was allowed to stand overnight at room temperature, extracted 10 times with 50-ml. portions of ether, and the combined ether extracts were concentrated to dryness. The residue was dissolved in 5 ml. of ethanol; 2 ml. of glacial acetic acid and 0.5 ml. of phenylhydrazine were added. The resulting phenylhydrazone melted at 176°. A mixed melting point with an authentic sample of the phenylhydrazone of *p*-hydroxybenzaldehyde showed no depression.

The quantitative determination of the formic acid produced on oxidation with periodate was performed in the manner described by Potter and Hassid.¹⁴ When 100 mg. (0.65 millimole) of *p*-hydroxymandelaldehyde in 150 ml. of water was treated with 140 mg. (0.65 millimole) of $NaIO_4$, only 14.3 mg. (0.31 millimole) of formic acid was released. However, upon treatment of same amount of the aldehyde with 350 mg. of $NaIO_4$, the expected amount (30.3 mg., 0.66 millimole) of formic acid was released.

Reaction of *p*-Hydroxycinnamic Acid Methyl Ester with HNO_3 .—To a solution of the ester (1 g., 0.0056 mole) in 15 ml. of dioxane was added a solution of $NaNO_2$ (0.4 g., 0.0056 mole) in 15 ml. of water; the mixture was acidified with 5 *N* hydrochloric acid to pH 2. Upon standing at room temperature for 4 hours, a yellow precipitate (0.65 g., yield 58%) separated. After recrystallization from ethanol, the product melted at 148°. Calcd. for $C_{10}H_8O_4N$ (223.2): C, 53.8; H, 4.1; N, 6.3. Found: C, 53.6; H, 3.8; N, 6.4. The same product was obtained when 0.2 g. of the ester was treated with 0.1 g. of $NaNO_2$ in 300 ml. of dilute aqueous hydrochloric acid (pH 2). Saponification of the product with 2 *N* alcoholic KOH gave an acid which melted at 223–225° after recrystallization from ethanol. Calcd. for $C_9H_7O_4N$ (209.1): N, 6.7. Found: N, 6.7. Johnson and Kohmann¹⁵ prepared 3-nitro-4-hydroxycinnamic acid and reported a melting point of 223° for the compound; they also reported a melting point of 142–144° for the methyl ester.

(14) A. L. Potter and W. Z. Hassid, *ibid.*, **70**, 3488 (1948).

(15) T. B. Johnson and E. F. Kohmann, *ibid.*, **37**, 166, 1876 (1915).

(13) G. D. Johnson, *THIS JOURNAL*, **73**, 5888 (1951).

NEW HAVEN, CONNECTICUT

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE RICE INSTITUTE]

Heats of Hydrogenation. I. Apparatus and the Heats of Hydrogenation of Bicyclo[2,2,1]heptene, Bicyclo[2,2,1]heptadiene, Bicyclo[2,2,2]octene and Bicyclo[2,2,2]octadiene

BY RICHARD B. TURNER, W. R. MEADOR AND R. E. WINKLER

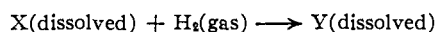
RECEIVED FEBRUARY 25, 1957

A calorimeter for the measurement of heats of catalytic hydrogenation in solution is described together with the results obtained for hydrogenations of bicyclo[2,2,1]heptene (–33.1 kcal./mole), bicyclo[2,2,1]heptadiene (–68.1 kcal./mole), bicyclo[2,2,2]octene (–28.3 kcal./mole) and bicyclo[2,2,2]octadiene (–56.2 kcal./mole) in acetic acid at 25°.

Some years ago Kistiakowsky¹ and his associates measured the heats of catalytic hydrogenation of a variety of unsaturated organic substances in the gas phase at 82°. The results obtained for the fifty-one compounds that were included in this investigation, have had important theoretical consequences of continuing interest. Since the gas phase technique is applicable only to substances possessing appreciable volatility, extension of the

(1) (a) G. B. Kistiakowsky, H. Romeyn, J. R. Ruhoff, H. A. Smith and W. E. Vaughan, *THIS JOURNAL*, **57**, 65 (1935); (b) G. B. Kistiakowsky, J. R. Ruhoff, H. A. Smith and W. E. Vaughan, *ibid.*, **57**, 876 (1935); (c) **58**, 137 (1936); (d) **58**, 146 (1936); (e) M. A. Dolliver, T. L. Gresham, G. B. Kistiakowsky and W. E. Vaughan, *ibid.*, **59**, 831 (1937); (f) M. A. Dolliver, T. L. Gresham, G. B. Kistiakowsky, E. A. Smith and W. E. Vaughan, *ibid.*, **60**, 440 (1938); (g) J. B. Conn, G. B. Kistiakowsky and E. A. Smith, *ibid.*, **61**, 1865 (1939). See also J. B. Conant and G. B. Kistiakowsky, *Chem. Revs.*, **30**, 181 (1937).

method to the liquid phase (29°) was undertaken in the Harvard laboratories by Williams,² and the heats of hydrogenation of nine additional compounds were determined by the latter procedure in 1942. Since that time no further experiments involving direct measurements of heats of hydrogenation have been reported, and in view of the considerable potential of the method, a continuation of this study appeared to be most desirable. The present paper constitutes the first report of work carried out in this Laboratory with the object of examining heat changes involved in reactions of the type



conducted in acetic acid solution at 25°.

(2) R. B. Williams, *THIS JOURNAL*, **64**, 1395 (1942).