(75 mL) and benzene (80 mL), and pyrrolidine (3.5 mL, 42 mmol) was added. This mixture was heated at reflux under nitrogen for 24 h. During the reflux period, the refluxing solvent was percolated through K_2CO_3. The reaction mixture was cooled, concentrated in vacuo to ~50 mL, and diluted with EtOAc (150 mL). This mixture was then extracted with 2 N NaOH (2 × 75 mL), 2 N HCl (3 × 75 mL), and brine (1 × 25 mL). The resulting organic layer was dried ($MgSO_4$) and solvent removed in vacuo to give a dark viscous oil. Silica gel chromatography (1 kg, 25% EtOAc/hexane) afforded 3.0 g (41%) of the desired product. The basic extract of the crude reaction mixture gave, upon acidification, 2.7 g of salicylamide. Based on recovered starting material the yield is calculated as 80%: mp 135 °C; IR (mull) 3180, 3080 (NH), 1680 (C=O), 1615, 1585 (C=C) 1260, 1150, 755 (C—O/other) cm^{-1}; NMR ($CDCl_3$) δ 8.2–7.9 (m, 2 H), 7.65–6.80 (m, 3 H), 1.66 (s, 6 H). Anal. Calcd for $C_{10}H_{11}NO_2$ (mol wt 177): C, 67.78; H, 6.25; N, 7.90. Found: C, 67.73; H, 6.23; N, 7.83.

Acknowledgment. The technical assistance of P. M. Gold is gratefully acknowledged.

Registry No. Salicylamide, 65-45-2; acetone, 67-64-1; 3-pentanone, 96-22-0; cyclopentanone, 120-92-3; cyclohexanone, 108-94-1; 4-*tert*-butylcyclohexanone, 98-53-3; tetrahydro-(4*H*)-thiopyran-4-one, 1072-72-6; 1-methyl-4-piperidinone, 1445-73-4; cycloheptanone, 502-42-1; cyclododecanone, 830-13-7; 1-phenylethanone, 98-86-2; 1-(3-thienyl)ethanone, 1468-83-3; benzaldehyde, 100-52-7; 5-carboxaldehyde-1,3-benzodioxole, 120-57-0; 3,4,5-trimethoxybenzaldehyde, 86-81-7; pentanal, 110-62-3; 2,3-dihydro-2,2-dimethyl-4*H*-1,3-benzoxazin-4-one, 30914-88-6; 2,3-dihydro-2,2-diethyl-4*H*-1,3-benzoxazin-4-one, 77773-92-3; spiro-[2*H*-1,3-benzoxazine-2,1'-cyclopentan]-4(3*H*)-one, 40033-94-1; spiro[2*H*-1,3-benzoxazine-2,1'-cyclohexan]-4(3*H*)one, 40033-95-2; 4'-(1,1-dimethylethyl)-spiro[2*H*-1,3-benzoxazine-2,1'-cyclohexan-4(3*H*)-one], 77773-93-4; 2',3',5',6'-tetrahydro-spiro-[2*H*-1,3-benzoxazine-2,4'-[4*H*]thiopyran]-4(3*H*)-one, 77773-94-5; 1'-methylspiro[2*H*-1,3-benzoxazine-2,4'-piperidin]-4-(3*H*)-one, 77773-95-6; spiro[2*H*-1,3-benzoxazine-2,1'-cycloheptan]-4-(3*H*)-one, 77773-96-7; spiro[2*H*-1,3-benzoxazine-2,1'-cyclododecan]-4(3*H*)-one, 77773-97-8; 2,3-dihydro-2-methyl-2-phenyl-4*H*-1,3-benzoxazin-4-one, 40033-93-0; 2,3-dihydro-2-methyl-2-(3-thienyl)-4*H*-1,3-benzoxazin-4-one, 77773-98-9; 2,3-dihydro-2-phenyl-4*H*-1,3-benzoxazin-4-one, 6629-80-7; 2,3-dihydro-2-(1,3-benzodioxol-5-yl)-4*H*-1,3-benzoxazin-4-one, 77773-99-0; 2,3-dihydro-2-(3,4,5-trimethoxyphenyl)-4*H*-1,3-benzoxazin-4-one, 52942-53-7; 2,3-dihydro-2-butyl-4*H*-1,3-benzoxazin-4-one, 77774-00-6.

Supplementary Material Available: Full experimental details for entries 3–17 in Table I (4 pages). Ordering information is given on any current masthead page.

Kinetics and Mechanism for *o*-Hydroxybenzaldehyde Phenylhydrazone Formation

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Received January 26, 1981

Benzaldehyde phenylhydrazone formation occurs with rate-determining carbinolamine formation under slightly acidic conditions and with rate-determining dehydration of the carbinolamine under neutral or basic conditions[2] (see eq 1 and 2). The addition of phenylhydrazine to form the

$$RNH_2 + C{=}O \underset{}{\overset{k_H}{\rightleftharpoons}} RNH{-}C{-}OH \underset{k_2}{\xrightarrow{k_1 a_H}} RN{=}C + H_2O \quad (1)$$

$$K_{ad} = \frac{[RNH{-}C{-}OH]}{[RNH_2][C{=}O]} \quad (2)$$

carbinolamine from this substrate is subject to both specific-acid and general-acid catalysis by carboxylic acid (α = 0.35). The dehydration step is subject to acid catalysis although pH-independent and base-catalyzed processes also occur.[2]

The rates of reaction of several ortho-substituted benzaldehydes, with substituents that are not capable of formation of a hydrogen bond with the carbonyl group, with phenylhydrazine have been studied.[3]

A detailed study of the kinetics of phenylhydrazone formation from *o*-hydroxybenzaldehyde was undertaken in order to examine the effects on reactivity toward nucleophiles of an ortho substituent capable of formation of a hydrogen bond with the oxygen of the carbonyl group of the aldehyde.

Experimental Section

Materials. All reagents employed were obtained commercially and, with exception of reagent grade inorganic salts, were either redistilled or recrystallized before use. Solutions of phenylhydrazine were prepared just prior to use.

Kinetics measurements were carried out spectrophotometrically at 25.0 °C with the aid of a Zeiss PMQ II spectrophotometer equipped with a cell holder through which water from a thermostated bath was continuously circulated. Reaction kinetics were monitored by observing the appearance of the phenylhydrazone at 347 nm, with an initial concentration of *o*-hydroxybenzaldehyde of 3.3×10^{-5} M. In all cases a sufficient excess of nucleophilic reagent was employed so that pseudo-first-order rate behavior was observed. First-order rate constants were evaluated from slopes of plots of log ($OD_\infty - OD_t$) against time in the usual manner.

It was difficult to determine spectrophotometrically the equilibrium constant for the formation of the carbinolamine from the *o*-hydroxybenzaldehyde and phenylhydrazine as a result of the strong light absorption of the latter substance. Similar difficulties have been noted in attempts to determine the equilibrium constants for the formation of other phenylhydrazine carbinolamines.[2,4] With *o*-hydroxybenzaldehyde the reaction is first order in phenylhydrazine concentration over the concentration range 1.0×10^{-3} to 5.0×10^{-3} M at pH 7. Consequently, all kinetic studies have been made by employing a phenylhydrazine concentration lower than 5.0×10^{-3} M. Second-order rate constants could therefore be determined by directly dividing first-order rate constants by the concentration of phenylhydrazine free base.

All kinetic experiments were carried out in 20% aqueous ethanol at an ionic strength of 0.50, maintained with KCl, with 2.0×10^{-4} M EDTA. Values of apparent pH were recorded with a Radiometer Model PHM 4d pH meter equipped with a glass electrode. Calculations of the concentrations of phenylhydrazine free base and of undissociated carboxylic acids were made employing the Henderson–Halsselbalch equation and values of pK_a from ref 3 and 5.

pK_a Determinations. The value of pK_a of *o*-hydroxybenzaldehyde was obtained at 25.0 °C in 20% aqueous ethanol and at an ionic strength of 0.50, maintained with KCl, with a Zeiss PMQ II spectrophotometer by measuring the effect of pH on the absorption of light at 379 nm (Table I, supplementary material). Plots of $E^- - OD/[H^+]$ against OD yield a pK_a of 8.42.

Results and Discussion

In Figure 1, logarithms of second-order rate constants for the reaction of phenylhydrazine with *o*-hydroxybenzaldehyde in 20% aqueous ethanol at 25.0 °C and an ionic strength of 0.50 are plotted as a function of pH. The curve

(1) Fellow of the Fundação de Amparo à Pesquisa do Estado de São Paulo.
(2) L. do Amaral and M. P. Bastos, *J. Org. Chem.*, **36**, 3412 (1971).
(3) M. P. Bastos and L. do Amaral, *J. Org. Chem.*, **44**, 980 (1979).
(4) W. P. Jencks, *J. Am. Chem. Soc.*, **81**, 475 (1959).
(5) R. Moscovici, J. P. Ferraz, E. A. Neves, J. O. Tognoli, M. I. El Seoud, and L. do Amaral, *J. Org. Chem.*, **41**, 4093 (1976).

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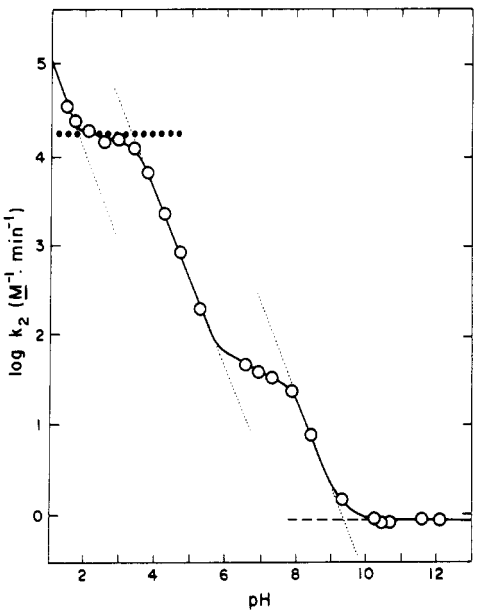


Figure 1. Logarithms of second-order rate constants for *o*-hydroxybenzaldehyde phenylhydrazone formation in 20% aqueous ethanol at 25.0 °C and an ionic strength of 0.50 plotted as a function of pH. The dotted lines indicate the rate constants for hydronium in the catalyzed process. The broken line indicates the rate constant for the uncatalyzed process. The open-circle line indicates the rate constant for internal catalysis (see text). Data have been taken from Tables II and III.

Table II. Catalytic Constants for Hydronium Ion Catalyzed (k_H) and for pH-Independent Reaction (k_0) for the Addition of Phenylhydrazine to Several Carbonyl Compounds in 20% Aqueous Ethanol at 25.0 °C and an Ionic Strength of 0.50

	k_H, M^{-2} min^{-1}	k_0, M^{-1} min^{-1}	ref
benzaldehyde	9.0×10^5	8.8×10^2	4
p-methoxybenzaldehyde	1.6×10^5	8.8×10^1	4
o-methoxybenzaldehyde	1.4×10^6	2.4×10^2	2
p-hydroxybenzaldehyde	1.7×10^5	6.6×10^1	4
o-hydroxybenzaldehyde	1.0×10^6	1.4×10^4	this work
acetophenone	2.3×10^4	6.2×10^{-1}	7
2′-hydroxyacetophenone	1.5×10^4	1.1×10^{-1}	7

shows two breaks, one near pH 3.5 and the second near pH 8. The former must reflect the transition in rate-determining step, and the latter, which occurs near the pK_a for the substrate, must reflect the loss of the phenolic hydrogen of the substrate.

In the regions of rate-determining carbinolamine formation for benzaldehyde or *o*-methoxybenzaldehyde phenylhydrazone formation, the second-order rate constants are sensitive functions of the nature and concentration of carboxylic acid–carboxylate buffers employed to maintain constant pH.[2,3] Studies of the buffer catalysis demonstrated that the catalysis is of the general-acid type.[4] In contrast, in regions of rate-determining carbinolamine formation for *o*-hydroxybenzaldehyde phenylhydrazone formation, general-acid catalysis by carboxylic acid–carboxylate was not observed on increasing the total buffer concentration (CNAcOH, ClAcOH, HCO_2H, $BrCH_2AcOH$, and AcOH) from 0.050 to 0.25 M, as observed with *o*-hydroxyacetophenone.[6]

Rate constants characterizing the various pathways for *o*-hydroxybenzaldehyde phenylhydrazone formation are collected in Tables II and III.

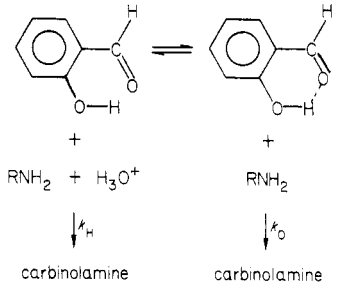
Table III. Catalytic Constants for Hydronium Ion Catalyzed ($K_{ad}k_1$) and Uncatalyzed Reaction ($K_{ad}k_2$) for the Dehydration of the Carbinolamine Intermediate in the Reaction of Phenylhydrazine with Several Benzaldehydes in 20% Aqueous Ethanol at 25.0 °C and an Ionic Strength of 0.50

substituent	$K_{ad}k_1$, M^{-2} min^{-1}	$K_{ad}k_2$, M^{-1} min^{-1}	ref
H	2.4×10^8	0.25	4
p-OCH_3	7.0×10^7	0.088	4
o-OCH_3	1.1×10^9	0.80	2
p-OH	3.0×10^7		4
o-OH	3.8×10^7		this work
o-O^-	2.1×10^9	0.88	this work

The reaction of phenylhydrazine with *o*-hydroxybenzaldehyde occurs with rate-determining carbinolamine formation at a pH below 3.5. This step shows specific acid-catalyzed and pH-independent reactions.

One sees from Table II that the rate of acid-catalyzed formation of the carbinolamine derived from the reaction of phenylhydrazine and *o*-hydroxybenzaldehyde is sixfold higher than that from *p*-hydroxybenzaldehyde, which is similar with to what is observed with the methoxybenzaldehydes.[2,3] On the other hand, the pH-independent formation of the carbinolamine derived from the reaction of phenylhydrazine and *o*-hydroxybenzaldehyde is 200-fold higher than the pH-independent reaction with *p*-hydroxybenzaldehyde.[2] This last ratio is very much higher than that observed with the methoxybenzaldehydes.[2,3]

An alternative explanation for the uncatalyzed reaction is that the reaction is subject to an internal catalysis by the intramolecular hydrogen bond between the hydrogen of the hydroxylic group on the ortho position and the oxygen of the carbonyl group.[7]



The internal catalysis explains the observation that the formation of the carbinolamine from phenylhydrazine and *o*-hydroxybenzaldehyde occurs without general-acid catalysis by carboxylic acids.

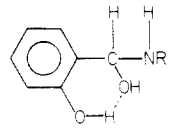
The introduction of a hydroxy group in the ortho position of the acetophenone decreases sixfold the value of the pH-independent formation of the corresponding carbinolamine.[6] while the introduction of a hydroxy group in the ortho position of the benzaldehyde increases 200-fold the pH-independent formation of the corresponding carbinolamine. These results can be discussed in terms of the effect of the methyl group linked to the carbonyl group of the acetophenone on the structure of the transition state. The formation of the carbinolamine intermediate is accompanied by a change in the hybridization of the carbonyl carbon from sp^2 to sp^3. The more crowded the carbinolamine is, the higher the energy of the incipient transition state. It is reasonable to expect that the transition state derived from the *o*-hydroxyacetophenone is more crowded than that of *o*-hydroxybenzaldehyde and that this crowd-

(6) K. B. Alves, M. P. Bastos, and L. do Amaral, *J. Org. Chem.*, 43, 4032 (1978).

(7) J. C. Dearden and W. F. Forbes, *Can. J. Chem.*, 38, 1837 (1960).

ing will affect the observed rate adversity.

The dehydration of the carbinolamine derived from the reaction of phenylhydrazine and *o*-hydroxybenzaldehyde, observed from pH 3.5 to 6, occurs with specific-acid catalysis but without general-acid catalysis by carboxylic acids, while general-acid catalysis by carboxylic acids has been observed in the dehydration of other carbinolamines.[8] It is possible to explain the absence of general-acid catalysis of the dehydration by an intramolecular acid catalysis by hydrogen bond.



Acknowledgment. The authors are indebted to Dr. Eugene H. Cordes for helpful comments concerning this work.

Registry No. *o*-Hydroxybenzaldehyde, 90-02-8; phenylhydrazine, 100-63-0; *o*-hydroxybenzaldehyde phenylhydrazone, 614-65-3.

Supplementary Material Available: Table I, determination of values of pK_a for *o*-hydroxybenzaldehyde at 25.0 °C in 20% aqueous ethanol and an ionic strength of 0.50 (1 page). Ordering information is given on any current masthead page.

(8) P. Sojo, F. Viloria, L. Malave, R. Possomani, M. Calzadilla, J. Baumrucker, A. Malpica, R. Moscovici, and L. do Amaral, *J. Am. Chem. Soc.* **98**, 4519 (1976).

(±)-[*methyl*-^{3}H and -^{2}H]Mianserin. Participants in a Dramatic Instance of HPLC Isotopic Fractionation[1]

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Received February 20, 1981

One functionality common to a number of valuable central nervous system (CNS) drugs both naturally occurring and synthetic is the *N*-methyl group. The availability of C^3H_3I at high specific activity (40–90 Ci/mmol) has allowed the preparation of *N*-[^{3}H]methyl CNS drugs at correspondingly high specific activity from accessible nor precursors for receptor binding studies.[2] In this way, we have prepared [*methyl*-^{3}H]oxymorphone (**1**), [*methyl*-^{3}H]morphine (**2**), [*methyl*-^{3}H]dihydromorphine (**3**), and [*methyl*-^{3}H]LSD (**4**) at high specific activity.[3] Although the identity of these substances has been conclusively demonstrated by spectroscopic (UV, ^{3}H NMR) evidence and by in vitro receptor binding assay, we have occasionally noted differences in their HPLC behavior as compared to cold standard. In particular, **2**–**4** have been observed to elute later than their respective cold standards on microporasil HPLC and we have attributed this behavior to isotopic fractionation.[4] To document the often intriguing HPLC behavior of these *N*-[^{3}H]methyl CNS drugs and thereby facilitate their identification, we now report the most dramatic instance of this phenomenon that we have yet observed.

(±)-Mianserin (**5a**) is an *N*-methyl tetracyclic antidepressant with affinity for both the serotonin and histamine receptors.[5] By use of either CNBr or vinyl chloroformate,[6]

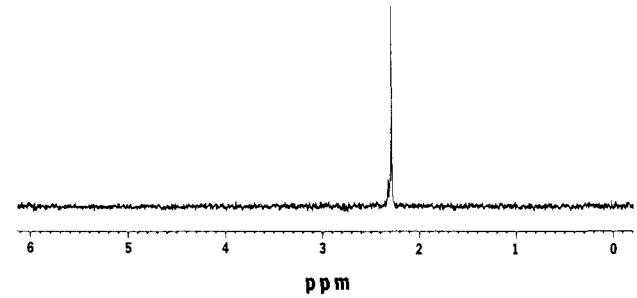


Figure 1. ^{3}H NMR spectrum of [*methyl*-^{3}H]mianserin (**5c**) in $CDCl_3$. Chemical shift values are in parts per million downfield from internal $(CH_3)_4Si$.

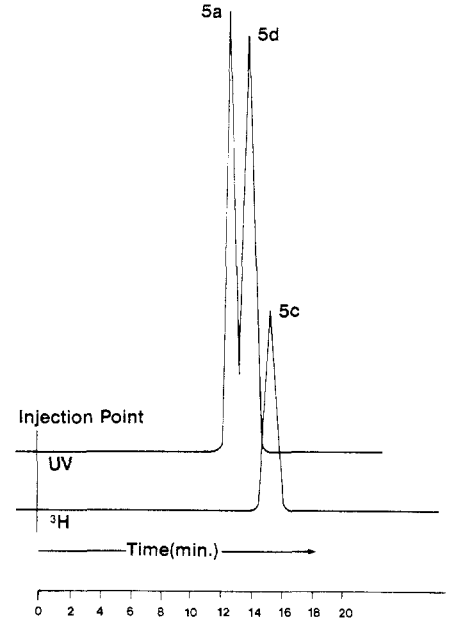
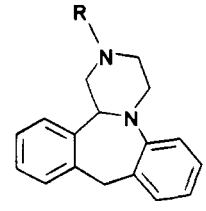


Figure 2. HPLC trace of compounds **5a**, **5d**, and **5c** coinjected on microporasil eluted at 1 mL/min with CH_2Cl_2–CH_3OH (98:2) and monitored by simultaneous UV (254 nm) and ^{3}H detection.

Table I. Field-Desorption Mass Spectral Data for 5c

m/e	% of base peak	species
264	8.8	cold mianserin
266	1.5	[*N*-C^{1}H$_2$^{3}H$_1$]mianserin
268	10.3	[*N*-C^{1}H$_1$^{3}H$_2$]mianserin
270	79.4	[*N*-C^{3}H$_3$]mianserin



5a R = CH_3
5b R = H
5c R = C^3H_3
5d R = C^2H_3

(1) Presented in part at the 11th Northeast Regional Meeting of the American Chemical Society, Rochester, NY, October 1981.
(2) Yamamura, H. I.; Enna, S. J.; Kuhar, M. J., Eds. "Neurotransmitter Receptor Binding"; Raven Press: New York, 1978.
(3) Filer, C. N.; Fazio, R.; Ahern, D. G., unpublished results.
(4) Klein, P. D. In "Advances In Chromatography"; Giddings, J. D., Keller, R. A., Eds.; Marcel Dekker: New York, 1966; Vol. 3, p 3.

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