Reactions of the Carbonyl Group with Nitroso Compounds. The Cases of Pyruvic Acid and Acetaldehyde

Stanko Uršić,* Viktor Pilepić, Valerije Vrček, Mario Gabričević and Branka Zorc
Faculty of Pharmacy and Biochemistry, University of Zagreb, 41000 Zagreb, A. Kovačića I, Croatia

Pyruvic acid and acetaldehyde react with substituted nitrosobenzenes to give the corresponding *N*-phenylacetohydroxamic acids. A mechanism for these reactions involving three sequential steps is proposed. The first step is the nucleophilic attack of the nitroso group on the carbonyl group, which leads to the formation of an unstable dipolar intermediate. This step is followed by proton transfer to the dipolar intermediate to form a more stable cationic intermediate, which, in the subsequent step, undergoes decarboxylation (in the case of pyruvic acid) or elimination of a proton from the carbon of the nitrosocarbinolic group (in the case of acetaldehyde), leading to the final product, hydroxamic acid.

The reaction of pyruvic acid includes an intramolecular reaction pathway, along with an acid-catalysed one. The experimental evidence obtained in support of such a description includes: (*a*) the order of reactivity of substituted nitrosobenzenes as demonstrated by the plot of log k_{obs} *vs.* Hammett parameters with slope −1.97 in the case of pyruvic acid and −0.93 in the case of acetaldehyde; (*b*) the observation of acid-catalysed and 'uncatalysed' pathways in the reaction of pyruvic acid; (*c*) the observation of general acid catalysis in these reactions; (*d*) the observation of an inverse solvent deuterium isotope effect of 0.41 in the case of acetaldehyde; (*e*) the observation of a solvent deuterium isotope effect of *ca.* 1.0 in the acid-catalysed reaction, and solvent isotope effect of *ca.* 1.2 in the 'uncatalysed' reaction of pyruvic acid with nitrosobenzene.

Pyruvic acid has an important role in many fundamental biochemical processes.[1] It is involved in the metabolism of amino acids and carbohydrates and in various other biosynthetic and biodegradative processes, where pyruvate serves as an important metabolic intermediate, both in aerobic and anaerobic metabolism.

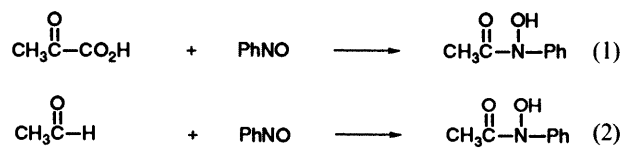
It has been reported that pyruvic acid and some α-keto acids are converted, by a thiamine-catalysed enzymatic reaction with nitrosobenzenes, into *N*-phenylhydroxamic acids.[2]

Recently, we have reported that pyruvic acid reacts with substituted nitrosobenzenes in aqueous acidic medium to give the corresponding *N*-phenylacetohydroxamic acids.[3]† Hydroxamic acids and their chemistry have received considerable attention[4–13] in connection with a variety of industrial and pharmaceutical applications, as well as their role as siderophores,[11–13] and as model systems for natural siderophores. Siderophores are low molecular weight multidentate ligands which serve as iron(III) ion-specific chelators of biological importance, and many naturally occurring siderophores are actually hydroxamic acids.

Now we report, along with results related to the reaction of pyruvic acid with nitrosobenzenes, the results of our investigation of the reaction of acetaldehyde with substituted nitrosobenzenes.‡

Results and Discussion

For reactions (1) and (2) spectroscopic evidence shows consumption of the nitroso compound (see Fig. 1). The formation of *N*-phenylacetohydroxamic acid was confirmed by the charac-



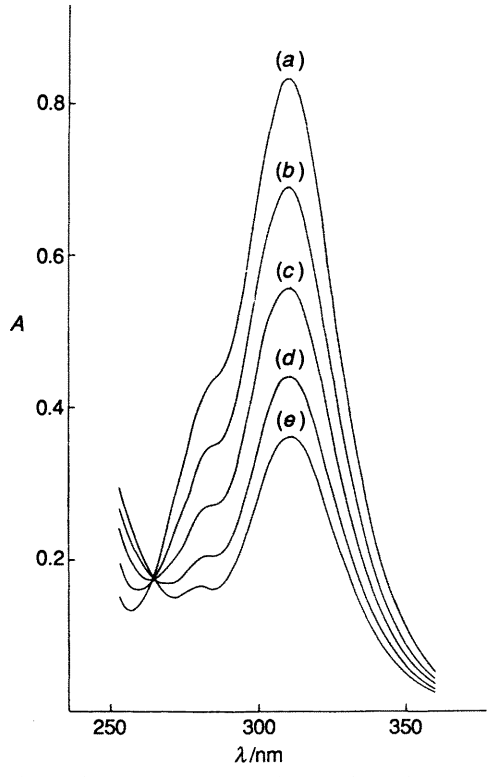


Fig. 1 Change in UV spectrum of the reaction mixture containing 0.200 mol dm^{-3} acetaldehyde, 10^{-4} mol dm^{-3} nitrosobenzene in presence of 0.5 mol dm^{-3} H_3O^+ and 3.5 mol dm^{-3} NaCl, at 25 °C. Scans: (*a*), 9 min; (*b*), 35 min; (*c*), 64 min; (*d*), 96 min; (*e*), 123 min.

† This communication is a preliminary account of a portion of the work presented herein.

‡ A qualitative observation of this reaction was reported previously,[14] without any kinetic or mechanistic details.

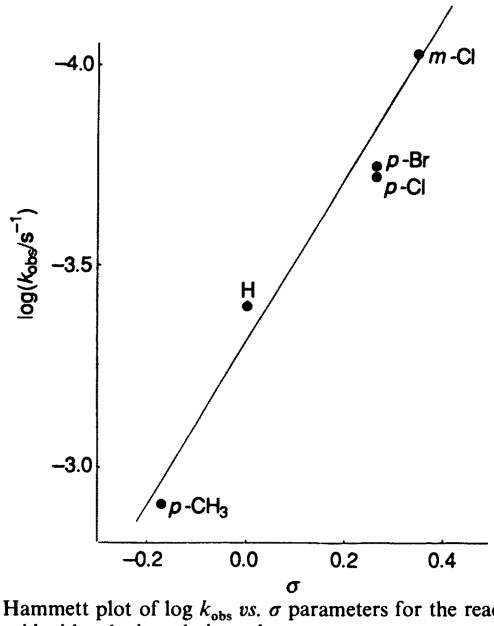


Fig. 2 Hammett plot of log k_{obs} *vs.* σ parameters for the reaction of pyruvic acid with substituted nitrosobenzenes

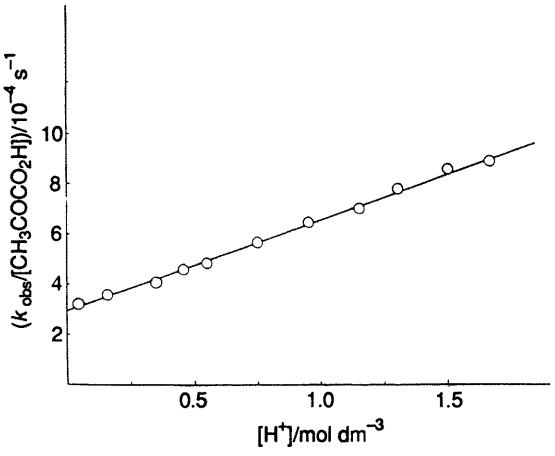


Fig. 3 Dependence of the rate of reaction of pyruvic acid with nitrosobenzene on concentration of H_3O^+. Data from Table 2.

terization of the isolated products, and by the spectra of the corresponding *N*-phenylacetohydroxamatoiron(III) complex. At constant hydrogen ion concentration, the pseudo-first-order rate constants for the formation of *N*-phenylacetohydroxamic acid depend linearly on the concentration of pyruvic acid in excess. Analogous results were obtained in the case of acetaldehyde. Therefore, both reactions should be of first order with respect to carbonyl compound, as well as with respect to nitrosobenzene, and both the reactions are second order overall. A linear plot (r = 0.988) of log k_{obs} *vs.* Hammett σ parameters with a slope of −1.97 was obtained for the reaction of pyruvic acid with substituted nitrosobenzenes (Fig. 2). In the case of acetaldehyde, the slope of the Hammett plot was −0.93 (straight line, r = 0.987). Here, k_{obs} follows from the experimentally obtained rate law: Rate = k[Py][H^+][PhNO] at constant [H^+].

The reaction of pyruvic acid with nitrosobenzene is acid-catalysed. The dependence of the quotient of observed rate constants and pyruvic acid concentration on hydrogen ion concentration [eqn. (3)] in the range 0.05–1.66 mol dm^{-3} is linear, with slope of 3.60 × 10^{-4} dm^6 mol^{-2} s^{-1}. The intercept of the straight line is 2.96 × 10^{-4} dm^3 mol^{-1} s^{-1}, which shows the existence of an additional, uncatalysed reaction path (see Fig. 3).

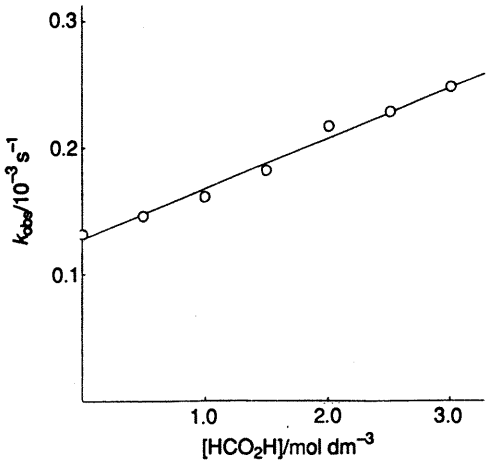


Fig. 4 Dependence of the rate of reaction of acetaldehyde with nitrosobenzene on the concentration of formic acid, in the presence of 0.094 mol dm^{-3} HCl at 25 °C and ionic strength 4.00 mol dm^{-3} (HCl/NaCl). Acetaldehyde: 1.00 mol dm^{-3}

The corresponding experimentally obtained equation is of the form of eqn. (3).

$$k_{obs}/[Py] = k[H^+] + k' \quad (3)$$

In the case of acetaldehyde, the observed rate constants depend linearly on the hydrogen ion concentration, having an intercept close to zero which indicates that the uncatalysed reaction may be neglected or perhaps does not exist at all under the conditions employed. The experimentally obtained rate-law is: Rate = k[CH_3CHO][H^+][PhNO].

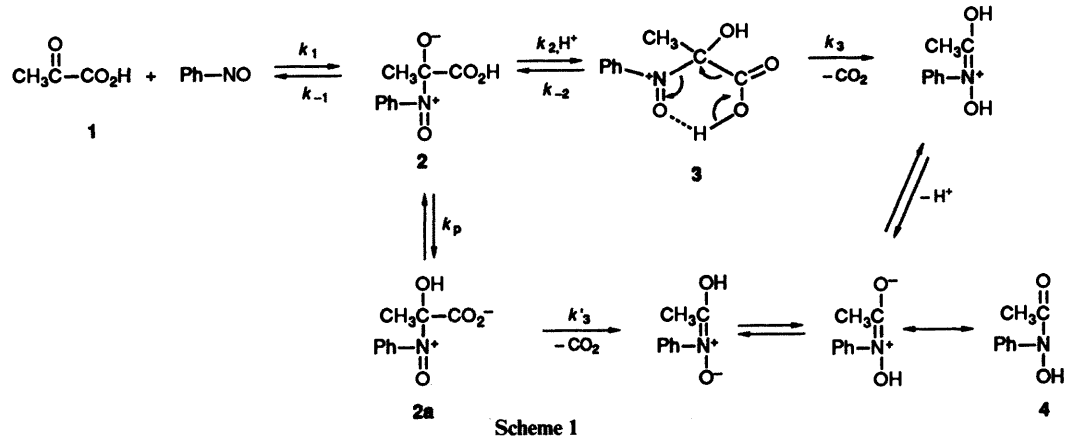
General acid catalysis with trichloroacetic acid was observed in the reaction of pyruvic acid with nitrosobenzene, and with formic acid in the case of acetaldehyde (Fig. 4). The rate constant for catalysis with formic acid is 30-fold smaller than the catalytic rate constant for the solvated proton, the ratio which is normally expected if the proton transfer process from an acid catalyst is diffusion-controlled.

The addition of neutral salt leads to an increase in the reaction rate in both reactions. In the case of the reaction of pyruvic acid with nitrosobenzene, the reaction rate increases linearly (r = 0.9996) with the concentration of added sodium perchlorate, in the range 0.40–5.24 mol dm^{-3}. Nonlinear dependence of the rate of the reaction on the added neutral salt was observed in the case of acetaldehyde, where log k_{obs} correlate linearly (r = 0.9998) with the concentration of the added sodium chloride in the range 1.06–5.00 mol dm^{-3}.

Solvent deuterium isotope effects $k(H_2O)/k(D_2O)$ were observed in both investigated reactions, but these effects in the two reactions differ considerably in magnitude and direction. The observed solvent deuterium isotope effects in the reaction of pyruvic acid with nitrosobenzene are small and normal, varying from *ca.* 1.20 at 0.12 mol dm^{-3} H_3O^+ to 1.03 at 1.16 mol dm^{-3} H_3O^+, while the corresponding observed solvent isotope effect in the reaction of acetaldehyde is *ca.* 0.41. The observed solvent deuterium isotope effects are listed in Table 1.

A mechanism consistent with the above observations for the reaction of pyruvic acid with nitrosobenzenes, is given by Scheme 1 while the proposed mechanism for reaction of acetaldehyde is described by Scheme 2.

Pyruvic Acid.—The first reaction step is considered to involve the formation of the tetrahedral dipolar intermediate **2**. This proposal is supported by the following evidence: (i) The order of reactivity of substituted nitrosobenzenes is that of electron-donating properties, as demonstrated by the plot of log k_{obs} *vs.*

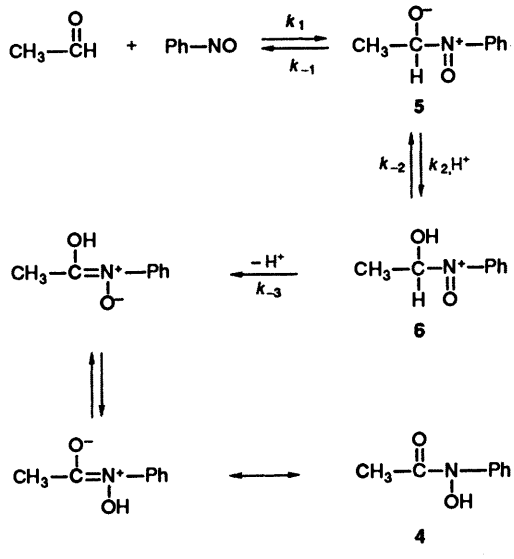


Scheme 1

Table 1 Solvent deuterium isotope effects in the reactions of pyruvic acid and acetaldehyde with nitrosobenzene[a]

Substrate	$[H_3O^+]$/ mol dm^{-3}	$\%D_2O$, (V/V)	$k(H_2O)/k(D_2O)$
Pyruvic acid[b]	0.121	98	1.15 (0.02)
	0.310	97	1.21 (0.07)
	1.164	94	1.03 (0.01)
Acetaldehyde	0.500	88	0.41 (0.01)[d]
	0.500	88	0.50 (0.02)[c]

[a] At 25 °C. Ionic strength 4.0 mol dm^{-3} (HCl/NaCl) in the case of acetaldehyde. No salt was added in the case of pyruvic acid. [b] From ref. 3. [c] Isotope effect when the reaction was initiated 22 h after the addition of acetaldehyde into heavy water. [d] Average of four paired experiments.



Scheme 2

Hammett σ parameters. This observation suggests the occurrence of nucleophilic attack of the nitroso group on the carbonyl carbon, leading to carbon–nitrogen bond formation and tetrahedral dipolar intermediate **2**. (ii) Carbon–nitrogen bond formation and the existence of a tetrahedral intermediate such as **2** are consistent with the structure of the product, hydroxamic acid. (iii) The formation of a tetrahedral intermediate is ordinarily the first step in many addition reactions to the carbonyl group.[15–18] (iv) In the similar reaction of glyoxylic acid with nitrosobenzenes,[19] and in the reaction of formaldehyde with nitrosobenzenes,[20] the occurrence of nucleophilic attack of the nitroso group on the carbonyl carbon was also proposed. (v) The observed acid catalysis can be accounted for by the existence of the dipolar addition intermediate, which would be trapped by proton transfer to form a more stable product, unless it reverted to reactants.[16,21]

The kinetic evidence obtained requires that (i) the reaction proceeds by both an acid-catalysed and, concurrently an uncatalysed reaction pathway, and (ii) the reaction starts from a molecule of pyruvic acid. These facts can be accounted for by the existence of two pathways for stabilisation of the highly unstable dipolar intermediate **2** by proton transfer in the second step, depicted in Scheme 1 as k_2 and k_P. The first pathway would be protonation of the dipolar intermediate **2** (or trapping of this intermediate by proton transfer from an acid catalyst where this process is thermodynamically favourable), to form a more stable cationic intermediate **3**.* The second pathway would be the intramolecular transfer of a carboxylic proton (perhaps also through a bridge of several water molecules[16,18]), to give the intermediate **2a**. The intermediates **3** and **2a** formed in the second step, undergo subsequent decarboxylation, giving the final product, hydroxamic acid.

The observed solvent deuterium isotope effect can be indicative of the structure of the cationic intermediate in the transition state for decarboxylation. In contrast to the case of acetaldehyde (see also below), the solvent isotope effects $k(H_2O)/k(D_2O)$ observed in the reaction of pyruvic acid with nitrosobenzene are normal and small, or close to unity (Table 1). The corresponding solvent deuterium isotope effect $k(H_2O)/k(D_2O)$ in the reaction of formaldehyde,[20] on the other hand, is 0.61, and 0.41 in the reaction of acetaldehyde. These inverse solvent isotope effects are related to the protonation of the dipolar addition intermediates in the second step of these reactions. The acid-catalysed reaction of pyruvic acid with nitrosobenzene is very similar to the reactions of acetaldehyde and formaldehyde, particularly with regard to the first and second reaction step. The solvent isotope effects observed in the reaction of pyruvic acid with nitrosobenzene seem therefore unexpected. These solvent isotope effects vary from *ca.* 1.2 at relatively low acidity, where the uncatalysed reaction prevails, to 1.0 at high acidity, where at least 60% of the reaction is acid catalysed (see Table 1). The cancellation of isotope effect with increasing hydrogen ion concentration, where the fraction of the acid-catalysed process is increased relative to the uncatalysed one, indicates that the component of the observed isotope effect pertinent to the acid-catalysed reaction should be inverse. However, the apparent magnitude of this effect, as compared with the observed solvent isotope effects in the analogous reactions of acetaldehyde and formaldehyde, is very different.

* In fact, this cationic intermediate is omitted, for simplicity, from Scheme 1, and **3** in Scheme 1 will serve to describe the transition state for decarboxylation of the cationic intermediate.

The kinetic evidence indicates, as mentioned above, that the carboxylic group is not deprotonated when decarboxylation is initiated. The decarboxylation should be rate-controlling in the reaction, since other processes in the reaction system are normally much more rapid than decarboxylation itself.* If the transfer of a carboxylic proton were a part of the reaction coordinate motion in the decarboxylation process, a sizeable isotope effect should result.[24] Our results show no significant primary deuterium isotope effect and, with regard to the expected inverse solvent isotope effect, it seems reasonable to conclude that mutual cancellation of the solvent isotope effect and primary deuterium isotope effect should have occurred. In order to explain our results, we have applied the concept proposed by Swain *et al.*[25] According to this concept, it is possible that proton transfer between electronegative elements accompanying heavy atom reorganization is not a part of the reaction coordinate motion. The proton then remains in a stable potential well during the cleavage of the carbon–carbon bond, and therefore a low isotope effect would result. If the transition state for decarboxylation is similar to **3**, having the proton in the stable potential well, which corresponds to the conditions proposed for the model of Swain *et al.*, the observed low isotope effect would not be unexpected. The mechanism of decarboxylation involving unimolecular decomposition through a cyclic transition state is, on the other hand, generally accepted for β-keto acids.[26] Intramolecular proton transfer as an integral part of the reaction mechanism was proposed for numerous other decarboxylation reactions.[22]

The observed solvent isotope effects at low proton concentration were related to the uncatalysed reaction, and we believe that the solvent isotope effect $k(H_2O)/k(D_2O)$ of 1.15 at 0.12 mol dm^{-3} H_3O^+ mainly reflects the solvent isotope effect on hydration of pyruvic acid.†‡

With regard to the uncatalysed reaction pathway it should be noted that there exists the possibility for another pathway§ to the intermediate **2a**. It was proposed [28–31] that pyruvic acid can exist in a strongly hydrogen bonded form with the hydrogen bond between the carboxylic proton and α-keto group ('proton chelate') even in water solution, in equilibrium with the 'unchelated' form. The formation of a dipolar addition intermediate renders feasible proton transfer down a hydrogen bond to the developing anionic oxygen. Such proton transfers down a hydrogen bond are known to be much faster than the rate of diffusion together of two reactants in ordinary aqueous solution.[32,33] Hence, intramolecular proton transfer down the hydrogen bond from the carboxylic group to the α-oxygen of the dipolar intermediate can compete with the diffusion-controlled trapping of this intermediate by a solvated proton. This process would lead to **2a**, along with the above described conversion of **2** to **2a**.

Assuming steady-state conditions for the system described by Scheme 1, the derived rate equation [eqn. (4)] will be entirely analogous to the experimentally obtained one [eqn. (3)] where

$$k_{obs} = k_3K_1K_2[Py][H^+] + k_3'K_1K_P[Py] \quad (4)$$

$K_1 = k_1/k_{-1}$ and $K_2 = k_2/k_{-2}$, revealing the possible meaning of the empirical parameters k and k'.

This reaction is much less sensitive toward the added organic cosolvent than the reaction of formaldehyde. The latter reaction is strongly retarded by the added organic cosolvent, as is the analogous reaction of glyoxylic acid.[2,14] In the reaction of formaldehyde the rate-controlling step is proton transfer from carbon,[20] while decarboxylation is expected to be rate-controlling in the reaction of pyruvic acid. Decarboxylation is known to be favoured by reducing the medium polarity, probably owing to changes in transition state solvation.[34–36]

Acetaldehyde.—Essentially, analogous experimental evidence and argumentation as in the case of pyruvic acid suggest that the first reaction step includes nucleophilic attack of the nitroso group on the carbonyl carbon and the formation of the dipolar intermediate **5** (Scheme 2). It seems reasonable to conclude that the observed acid catalysis and solvent deuterium isotope effect are consistent with proton transfer to the dipolar intermediate **5**, leading to the cationic intermediate **6**. Generally, trapping of an intermediate such as **5** by proton transfer from an acid catalyst would lead to acid catalysis.[16] The observed ratio of about 30, between the catalytic rate constants for hydronium ion and formic acid is consistent with the expected one for general acid catalysis by trapping where the proton transfer is diffusion-controlled.[16,17]

The observed inverse solvent deuterium isotope effect $k(H_2O)/k(D_2O)$ is 0.41 (in 89% deuterium oxide). This value leads to the reasonable estimated one of 0.31 for $k(H_2O)/k(D_2O)$, taking into account a solvent isotope effect $K(H_2O)/K(D_2O)$ of 0.86 for hydration of acetaldehyde.[27] An inverse solvent deuterium isotope effect is normally expected if there is pre-equilibrium protonation preceding a slow step in a complex reaction. The solvent deuterium isotope effects $K(H_2O)/K(D_2O)$ arising from an equilibrium protonation are usually 0.50–0.25.[37] The observed solvent isotope effect in this reaction should therefore be consistent with the preequilibrium protonation of the dipolar intermediate **5** in the second reaction step.

The second step is followed by the elimination of a proton from the carbon of the nitrosocarbinolic cation intermediate **6**. This process leads to the final product, hydroxamic acid. Probably, this process is rate controlling, since (i) proton transfer from carbon is ordinarily slow in comparison with other processes, *i.e.* proton transfer between electronegative atoms[23] and the formation and breakdown of the dipolar intermediates,[16–18] (ii) the analogous proton transfer from the carbon of nitrosocarbinolic cation intermediate in the closely similar reaction of formaldehyde with nitrosobenzenes is rate controlling for this reaction as demonstrated earlier.[20,¶]

With regard to the observed catalysis by hydronium ion and the observed solvent deuterium isotope effect, an alternative

* Decarboxylation is ordinarily a relatively slow process,[22] while proton transfers between electronegative atoms are fast processes.[23] Additions to carbonyl group are also much faster than decarboxylation as well as the breakdown of dipolar intermediate to reactants.[16–18,22]

† Generally, any solvent isotope effect observed in the reactions of strongly hydrated carbonyl compounds in aqueous solution should be complicated by a solvent isotope effect on hydration. The solvent isotope effect observed here in the uncatalysed reaction of pyruvic acid is closely similar in magnitude to the solvent isotope effect for hydration of acetaldehyde.[27]

‡ A referee has suggested that the difference between kinetic isotope effects in the solutions of slightly lower hydronium ion concentration may be insignificant.

§ Also, the intramolecularly catalysed ('uncatalysed') reaction is kinetically indistinguishable from another one, where pyruvate anion would react with nitrosobenzene. However, it seems that the 'uncatalysed' reaction is rather the one described by Scheme 1. Pyruvic acid is more reactive toward nucleophiles than its anion, and the intermediate which would arise from pyruvate anion and nitrosobenzene should be less stable than **2a**.

¶ In the reaction of acetaldehyde, a diminished solvent isotope effect was observed when the reaction was initiated for 22 h, after addition of acetaldehyde into heavy water (Table 1). Probably, this is a result of partial H/D exchange on the carbonyl carbon, which would lead to the primary isotope effect and partial cancellation of the inverse solvent deuterium isotope effect.

reaction path leading to the cationic intermediate **6** is possible. This reaction path would include formation of the intermediate **6** as a result of nucleophilic attack of nitrosobenzene on the protonated acetaldehyde, and cannot be kinetically distinguished from reaction *via* dipolar intermediate **5**. The observed general acid catalysis suggests the occurrence of the reaction *via* the dipolar intermediate, but does not rule out a possible concurrent reaction *via* the protonated aldehyde. The observed solvent isotope effect can also be related to the reaction *via* the protonated aldehyde. The question of the existence of this alternative reaction so far remains unresolved.

The rate of the reaction of acetaldehyde with nitrosobenzene is 7.71×10^{-5} dm^6 mol^{-2} s^{-1} (at zero ionic strength). Considering this rate constant as the rate of reaction *via* a dipolar intermediate, the corresponding rate constant for the reaction of formaldehyde[20] would be greater by a factor of 4.2×10^5. This great difference in the rates of the two reactions could be a consequence of the different rates of proton transfer from the carbon of cationic intermediates and the different stability of the dipolar intermediates. The steady-state treatment gives for the reaction system of Scheme 2 rate equation (5)

$$k_{obs} = k_3K_1K_2[CH_3CHO][H^+] \quad (5)$$

(where $k_1/k_{-1} = K_1$ and $k_2/k_{-2} = K_2$), analogous to the experimentally obtained one.

Experimental

Reagents and Apparatus.—Nitrosobenzenes were prepared by literature methods.[38] Sodium pyruvate (Fluka, puriss, > 99%) was used. Heavy water was from Fluka, 99.8%. Iron(III) perchlorate solutions were prepared according to the procedure in ref. 39. A stock solution of acetaldehyde was prepared by dissolving pure acetaldehyde (Fluka, min. 99.9%) in the appropriate amount of cold (0 °C) water. All other chemicals employed were of analytical grade purity. A Pye Unicam Model SP-8-100 spectrophotometer and a Beckmann 24 spectrophotometer, both equipped with thermostatted cell compartments were used for collection of kinetic data and for UV–VIS scans.

Synthesis of N-*Phenylacetohydroxamic Acid.—Method* (*A*). To a solution of nitrosobenzene (0.30 g, 0.003 mol) in 10 cm^3 of acetic acid (99.6%) and 3.3 cm^3 of 1.88 mol dm^{-3} HCl, a solution of sodium pyruvate (0.33 g, 003 mol) in 25 cm^3 water was added dropwise. The reaction mixture was stirred for 2.5 h at ambient temperature and extracted with chloroform (4×50 cm^3). The combined extracts were washed with water, dried and evaporated to give 0.36 g (80%) of *N*-phenylacetohydroxamic acid (*N*-phenyl-*N*-hydroxyacetamide). Recrystallization from light petroleum gave pale needles, m.p. 66–67 °C (lit.,[41] 67–67.5 °C). ν_{max}(KBr)/cm^{-1} 3140, 2880, 1625, 1585, 1475. λ_{max}/nm 250 (EtOH). λ_{max} of the monohydroxamatoiron(III) complex (in $CHClO_4/NaClO_4$) was 505 nm (lit.,[40] 505 nm).

Method (*B*). To a solution containing 40.00 g NaCl in 200 cm^3 water was added 2 cm^3 of concentrated hydrochloric acid and then 5 cm^3 of a 44% aqueous solution of acetaldehyde followed by a dropwise addition of a solution of nitrosobenzene in ethanol (0.30 g in 30 cm^3). The reaction mixture was stirred at room temperature for 20 h and then extracted with chloroform (4×100 cm^3). The combined extracts were washed with water, dried over anhydrous sodium sulfate and evaporated. Recrystallization from light petroleum gave the product with identical spectral and physical properties as in Method (A).

Kinetics.—Method (*A*). Most kinetics were performed by following the appearance of the absorbance of the mono-*N*-phenylacetohydroxamatoiron(III) complex at 520 nm. Usually, kinetics were initiated by addition of a solution of nitrosobenzene in methanol (0.02 cm^3) to the reaction mixture (2.5 cm^3, in a well stoppered and thermostatted silica cell), containing reactants and 5×10^{-3} mol dm^{-3} iron(III) ions [iron(III) perchlorate]. Pyruvic acid and proton concentration exceeded 100–3000 times that of nitrosobenzene. Rate constants were computed using a nonlinear least-squares fitting program, and good first-order kinetics ($r > 0.9997$) were obtained over more than four half-lives. Essentially the same procedure was performed for determining rate constants of the reaction of acetaldehyde with nitrosobenzenes, obtaining also good pseudo-first-order kinetics over at least four half-lives. In this case, ionic strength was usually 3.0 or 4.0 mol dm^{-3} (NaCl/HCl), and total concentration of acetaldehyde 1.0 mol dm^{-3}.

Method (*B*). Some kinetics (denoted with *c* in Table 2) were performed by sampling the reaction mixture without added ferric ions, at appropriate time intervals, to avoid catalytic reaction with iron(III). This method appeared to be necessary where the ratio H^+/Fe^{3+} was less than 50, and was also used as a control method in relation to Method (A). The samples of the reaction mixture (0.1 cm^3) were quickly mixed with iron(III) ion solution (0.01 mol dm^{-3} in 0.1 mol dm^{-3} $HClO_4$) and the absorbance of the hydroxamatoiron (III) complex was immediately measured. Other details were as for Method (A). Good kinetics, in agreement with those of Method (A), within the limits of experimental error, were obtained.

Method (*C*). This method was based on measuring the disappearance of the absorbance of the nitroso compound at 370 nm, and served only as a control method (*e* in Table 2). The results are in very good agreement with those of Methods (A) and (B).

Determination of the Dependence of the Rate of Reaction of Pyruvic Acid with Nitrosobenzene on Hydronium Ion Concentration.—Fig. 3 and Table 2 show the dependence of k_{obs}/[Py] on the hydronium ion concentration. Rates are corrected for

Table 2 Dependence of the rate of reaction of pyruvic acid with nitrosobenzene on the hydrogen ion concentration[a,b]

Run	$[H_3O^+]$/mol dm^{-3}	$k_{obs}/10^{-4}$ s^{-1}	$k^d_{corr}[Py]^{-1}/10^{-4}$ dm^3 mol^{-1} s^{-1}
1	0.049	1.92 (0.03)[c]	3.14
2	0.153	2.97 (0.09)[e]	
3	0.158	2.95 (0.01)[c]	3.54
4	0.161	3.11 (0.02)	
5	0.354	3.61 (0.15)[c]	
6	0.357	3.75 (0.10)	4.08
7	0.459	4.36 (0.04)	4.59
8	0.555	4.78 (0.04)	4.82
9	0.754	6.04 (0.04)	5.67
10	0.953	7.32 (0.17)	6.45
11	1.153	8.66 (0.12)	7.18
12	1.303	9.40 (0.27)	7.50
13	1.503	11.07 (0.14)	8.41
14	1.663	11.80 (0.10)	8.74

[a] At 25 °C. Ionic strength was 0.4 mol dm^{-3} ($HClO_4/NaClO_4$) up to the concentration of H_3O^+ of 0.354 (Runs 1–5). Ionic strength was not constant in other experiments (Runs 6–13). Concentrations of hydronium ion are total concentration of this ion, taking into account dissociation of pyruvic acid. The pK_a of pyruvic acid is taken (see ref. 42) to be 1.5. [b] Rate constants were determined as noted (see text), and are average of 3–5 runs. [c] Rate constants determined by the sampling method (see text), without addition of iron(III) ions to the reaction mixture. [d] Rate constants obtained from k_{obs}, after correction for the influence of ionic strength, where ionic strength exceeded 0.4 mol dm^{-3}. Concentrations of undissociated pyruvic acid are calculated as denoted above. [e] Rate constant obtained by the measuring of disappearance of absorbance of the nitroso compound at 370 nm.

the influence of ionic strength, in all cases where ionic strength exceeded 0.4 mol dm^{-3}. The added electrolyte increased the rate of the reaction of pyruvic acid with nitrosobenzene. We observed a linear dependence in the case of added $NaClO_4$, in the range 0.4–5.2 mol dm^{-3}. We have assumed that the effects of added $HClO_4$ (apart from the catalytic effect of hydronium ion) on the rate are not very different (at least in the range 0.4–1.6 mol dm^{-3}) from that of $NaClO_4$. Following this assumption we calculated the values of the rate constants at ionic strength of 0.4 mol dm^{-3}. The calculation was based on the empiric equation now rewritten in the form $(3.45 = k_0)$, $k_{obs} = k_0 (1 + 1.12/k_0 I)$, and, taking $k = k_{obs}$ at 0.4 mol dm^{-3} ionic strength and $\Delta I = (I - 0.4)$, it follows that $k_{obs} = k (1 + 0.30 \Delta I)$.

Acknowledgements

Financial support from the Croatian Research Council is gratefully acknowledged.

References

1 See for example: R. K. Murray, D. K. Grauner, P. A. Mayes, and V. W. Rodwell, *Harper's Biochemistry*, 22nd Edn., Appleton and Lange, East Norwalk, 1990, Sect. 2.
2 (*a*) M. D. Corbett and B. R. Chipko, *Bioorg. Chem.*, 1980, **9**, 273; (*b*) M. D. Corbett, D. R. Doerge and B. R. Corbett, *J. Chem. Soc., Perkin Trans. 1*, 1983, 765 and references cited therein.
3 S. Uršić, V. Vrček, M. Gabričević and B. Zorc, *J. Chem. Soc., Chem. Commun.*, 1992, 265.
4 M. J. Miller, *Chem. Rev.*, 1989, **89**, 1536.
5 E. Lipczynska-Kochany, *Chem. Rev.*, 1991, **91**, 477.
6 V. J. Robinson, P. J. Coles, R. A. Smith and A. Krantz, *J. Am. Chem. Soc.*, 1991, **113**, 7760.
7 E. K. Dolence and M. J. Miller, *J. Org. Chem.*, 1991, **56**, 492.
8 M. Izquierdo-Martin and R. L. Stein, *J. Am. Chem. Soc.*, 1992, **114**, 325.
9 J. H. Musser, A. F. Kreft, R. H. W. Bender, D. M. Kubrak, D. Grimes, R. P. Carlson, H. M. Hand and J. Chang, *J. Med. Chem.*, 1990, **33**, 240.
10 K. Yamamoto, Y. Shinoki, M. Nishio, Y. Matsuda, Y. Inouye and S. Nakamura, *J. Antibiotics*, 1990, **43**, 1012.
11 B. F. Matzanke, G. Muller-Matzanke and K. N. Raymond, *Siderophore Mediated Iron Transport: Chemistry, Biology and Physical Properties*, in *Physical Bioinorganic Chemistry*, eds. M. L. Loehr, H. B. Gray and A. B. P. Lever, VCH Publishers, New York, 1989.
12 A. L. Crumbliss, *Coord. Chem. Rev.*, 1990, **105**, 155.
13 R. C. Hider and A. D. Hall, in *Perspectives on Bioinorganic Chemistry*, eds. R. W. Hay, J. R. Dilworth and K. B. Nolan, JAI Press, London 1991, vol. 1, pp. 209–255.
14 M. Strah, S. Uršić and B. Zorc, *Croat. Chem. Acta*, 1989, **62**, 529.
15 T. H. Lowry and K. Schueller Richardson, *Mechanism and Theory in Organic Chemistry*, Harper and Row Publishers, New York, 1987, ch. 8.
16 W. P. Jencks, *Acc. Chem. Res.*, 1976, **9**, 425.
17 J. M. Sayer, B. Pinsky, A. Schonbrunn and W. Washtien, *J. Am. Chem. Soc.*, 1974, **96**, 7998.
18 S. Rosenberg, S. M. Silver, J. M. Sayer and W. P. Jencks, *J. Am. Chem. Soc.*, 1974, **96**, 7986.
19 M. D. Corbett and B. R. Corbett, *J. Org. Chem.*, 1980, **45**, 2834.
20 O. Kronja, J. Matijević-Sosa and S. Uršić, *J. Chem. Soc., Chem. Commun.*, 1987, 463.
21 W. P. Jencks, *Acc. Chem. Res.*, 1980, **13**, 161.
22 G. E. Dunn, *Isot. Org. Chem.*, 1977, **3**, 1, and references cited therein.
23 J. E. Crooks in *Proton Transfer Reactions*, eds., E. Caldin and V. Gold, Chapman and Hall, London 1975, ch. 6.
24 R. A. More O'Ferrall in ref. 23, ch. 8.
25 C. G. Swain, D. A. Kuhn and R. L. Schowen, *J. Am. Chem. Soc.*, 1965, **87**, 1553; R. L. Schowen, *Prog. Phys. Org. Chem.*, 1972, **9** 275.
26 M. W. Logue, R. M. Pollack and V. P. Vitullo, *J. Am. Chem. Soc.*, 1975, **97**, 6868.
27 G. E. Lienhard and W. P. Jencks, *J. Am. Chem. Soc.*, 1966, **88**, 3982.
28 A. Schellenberger and L. Oehme, *Z. Phys. Chem. (Leipzig)*, 1964, **227**, 112; L. Oehme and A. Schellenberger, *Chem. Ber.*, 1968, **101**, 1499.
29 G. Fischer, L. Oehme and A. Schellenberger, *Tetrahedron*, 1971, **27**, 5683.
30 J. F. Arnott, D. B. Larson and S. P. McGlynn, *J. Am. Chem. Soc.*, 1973, **95**, 7599.
31 W. J. Ray, J. F. Katon and D. B. Phillips, *J. Mol. Struct.*, 1981, **74**, 75.
32 M. Eigen, *Angew. Chem., Int. Ed. Engl.*, 1964, **3**, 1.
33 J. R. Escabi-Perez and J. H. Fendler, *J. Am. Chem. Soc.*, 1978, **100**, 2234.
34 D. S. Kemp and K. G. Paul, *J. Am. Chem. Soc.*, 1975, **97**, 7305.
35 J. F. Marlier and M. H. O'Leary, *J. Am. Chem. Soc.*, 1986, **108**, 4896.
36 M. H. O'Leary, *Acc. Chem. Res.*, 1988, **21**, 450.
37 P. M. Laughton and R. E. Robertson, in *Solute–Solvent Interactions*, eds. J. F. Coetzee and C. D. Ritchie, Marcel Dekker, New York, 1969, p. 399.
38 (*a*) A. I. Vogel, *Practical Organic Chemistry*, Longmans, Green and Co., London, 1951, p. 601; (*b*) W. J. Mijs, G. C. Hoekstro, R. M. Ulmann and E. Havinga, *Recueil*, 1958, **77**, 746.
39 C. P. Brink and A. L. Crumbliss, *Inorg. Chem.*, 1984, **23**, 4708.
40 B. Monzyk and A. L. Crumbliss, *J. Am. Chem. Soc.*, 1979, **111**, 6203.
41 E. Bamberger and H. Destraz, *Chem. Ber.*, 1902, **35**, 1874.
42 N. Hellström and S. O. Almquist, *J. Chem. Soc. B*, 1970, 1396.

Paper 2/05229F
Received 29*th September* 1992
Accepted 11*th November* 1992