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Hydroxamic Acids from the Reaction of Active Acetaldehyde with Aromatic Nitroso Compounds

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α-Hydroxyethylthiamine (**4**) reacts with nitrosobenzene (**5**) to produce *N*-phenylacetohydroxamic acid (**7**). A mechanism for this reaction is proposed. The possibility that similar reactions can occur *in vivo* with the production of carcinogenic hydroxamic acids is discussed.

In recent years considerable interest has been given to the hydroxamic acid functional group as an intermediary of carcinogenesis by certain aromatic amines (*1*). The metabolic *N*-hydroxylation of amines and amides by mixed function oxidases in mammalian liver is now considered as an essential activating step in carcinogenesis by these compounds. Most research has centered on the metabolic activation of 2-fluorenylacetamide (**1**) in several mammalian species to give *N*-hydroxy-2-fluorenylacetamide (**2a**) by *N*-hydroxylation. This essential activation to give a hydroxamic acid is followed by conjugation of the hydroxamate hydroxyl with sulfate, acetate, or other appropriate conjugate, which produces a facile leaving group on the amide nitrogen (*1*, *2*). It is

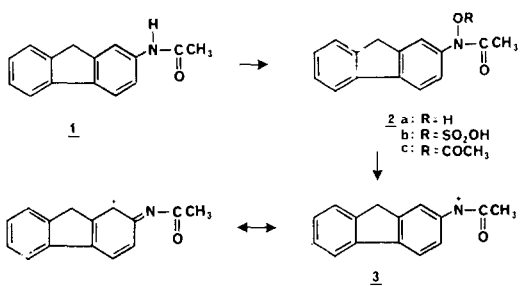

FIG. 1.

thought that the spontaneous decomposition of derivatives such as **2b** or **c** produces a nitrenium ion (**3**), which is a potent arylating reagent and which produces the biochemical lesion necessary to induce malignancy (*1*, *3*). Similar activation mechanisms have been proposed for other carcinogenic amines and amides. All the proposed mechanisms require *N*-hydroxylation of nitrogen by mixed function oxidases, which are limited for the most part to liver microsomes. Thus, hepatic tumors are most frequently observed, although tumors have been reported in organs other than the liver from the carcinogenic amines and amides investigated.

In our laboratories we have been investigating the possibility that hydroxamic acids could also be produced *in vivo* by nonmicrosomal metabolic reactions. This paper reports a previously unknown organic reaction that leads to a hydroxamic acid in a manner paralleling known biochemical reactions. The hydroxamic acid formed in the present study is not a known carcinogen, but there is no reason to doubt that similar reactions with formation of known carcinogens from appropriate nitroso compounds would occur. The present reaction should serve as a safe and convenient model for further study.

α-Hydroxyethylthiamine (**4**), as the phosphate ester, has been shown to be an intermediate in the conversion of pyruvic acid to acetyl coenzyme A, and is referred to as "active acetaldehyde" (*4*). This thiamine derivative and others have been shown to undergo nucleophilic reactions with resultant transfer of the α-hydroxyethyl group to a suitable carbonyl acceptor and liberation of thiamine. We found that the reaction of a α-hydroxyethylthiamine (**4**) with nitrosobenzene (**5**) produced a moderate yield of *N*-phenylacetohydroxamic acid (**7**). On the basis of Breslow's mechanisms for thiamine-catalyzed bioorganic reactions (*5*, *6*), we propose the mechanism illustrated in Fig. 2 for this reaction. The base-catalyzed nucleophilic addition of α-hydroxyethylthiamine (**4**) to the nitroso group in **5** parallels the nucleophilic addition of α-hydroxyethylthiamine to carbonyl compounds such as acetaldehyde (*7*) or pyruvic acid (*8*). The proposed reaction intermediate **6** would be expected to decompose in the manner indicated to produce the hydroxamic acid **7** and thiamine.

The reaction in Fig. 2 was carried out by dissolving α-hydroxyethylthiamine hydrochloride (*9*) (**4**) and a 1- to 5-fold amount of nitrosobenzene (**5**) in a polar solvent such as

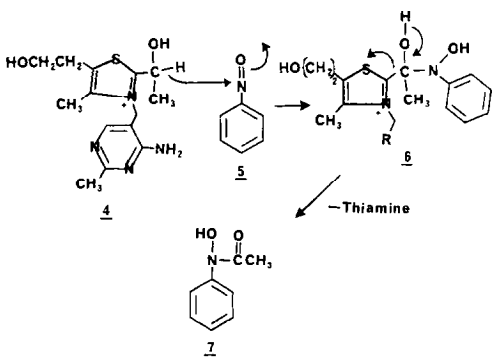

FIG. 2.

aqueous ethanol or dimethylformamide. Gentle heating under N_2 for several hours after the addition of 1–3 equiv of triethylamine sufficed for bringing the reaction to completion as determined by tlc analysis. Tlc analysis was used to follow the production of **7** and other side products, in addition to monitoring the disappearance of **4** and simultaneous formation of thiamine. The reaction was considered complete when all of **4** had disappeared. By varying the ratio of reactants and amount of triethylamine employed, the conditions favoring the greatest production of **7** in the solvent dimethylformamide were determined and are reported in the experimental section. Azoxybenzene and thiochrome were side products formed to the greatest extent from the

known decomposition of nitrosobenzene (*10*) and thiamine derivatives (*11*), respectively. Aqueous ethanol solvents resulted in the greatest amount of decomposition of reactants and thus lower yields of **7**.

The only related reaction that has appeared in the chemical literature is that reported between nitrosobenzene (**5**) and 3-ethyl-2-methylbenzothiazolium chloride (**8**), which led to the production of the nitrone **9** as shown in Fig. 3 (*12*). Such a reaction indicates that a carbon atom attached to the 2-position of a thiazolium salt is a sufficiently reactive nucleophile to condense with a nitroso group. Evidently the intermediate hydroxylamine was oxidized to the nitrone 9 under the conditions of the reaction. On the basis of our findings, we would expect that substitution of hydroxyl for one of the α hydrogens of **8** would produce **10**, although no such investigation has been attempted.

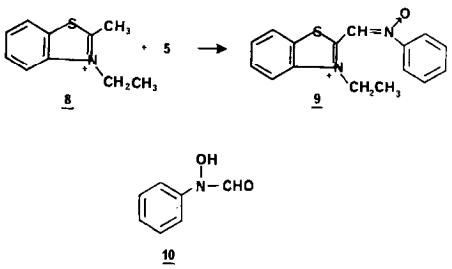

FIG. 3.

Baumgarten reported the oxidative cleavage of **7** with lead tetraacetate to give **5** (*13*), which is overall just opposite to the present reaction. There is no similarity in possible mechanisms between these two reactions, however.

In view of the present report, it becomes necessary to consider the possibility that carcinogenic hydroxamic acids can be formed from aromatic nitroso compounds by a process not requiring the intervention of microsomal oxidases. Since nitroso compounds are common blood metabolites of many amines (*14*), a potentially new metabolic route to toxic metabolites of amines and amides becomes evident. The requirement for active acetaldehyde in the proposed metabolic route is established from the known ubiquitous distribution in living systems of thiamine-dependent enzymes and of active acetaldehyde unbound to enzymes (*15*). The ability of aromatic nitroso compounds to compete with normal substrates for thiamine-dependent enzymes, such as ketolases, has yet to be demonstrated, although they are known to compete with carbonyl groups for the enzyme alcohol dehydrogenase (*16*). Once this is known, then an assessment can be made as to the importance of this proposed reaction to the toxic effects, including carcinogenicity, of certain aromatic amines.

EXPERIMENTAL SECTION

Melting points were taken on a calibrated Thomas–Hoover melting-point apparatus. Infrared spectra were obtained on a Beckman IR-33 and mass spectra on a DuPont 21-492 mass spectrometer. Column chromatography was performed on

silica gel 0.05–0.2 mm (E. Merck) and thin-layer chromatography (tlc) on precoated 0.25-mm silica gel F-254 plates (E. Merck) obtained from Brinkmann Instruments. Visualization of thin-layer chromatograms was achieved by viewing under a Mineralight UVSL-13 lamp. Spray reagents for further visualization were 1% $FeCl_3$ in 0.1 *M* HCl for hydroxamic acids and thiochrome reagent for thiamine derivatives (*17*).

Analytical Methods

Each reaction was analyzed in a semiquantitative manner by tlc to determine hydroxamic acid production and formation of thiamine. Aliquots of 0.2 ml were obtained every 30 min and 5 μl of each aliquot were chromatographed in two solvent systems on separate tlc plates. Plates chromatographed for 10 cm in 10% MeOH/$CHCl_3$ were visualized with the $FeCl_3$ spray, while plates chromatographed in CH_3CN/H_2O [4:1; HCO_2H to pH 2.5] (*18*) were sprayed with alkaline ferricyanide to detect thiamine. Each reaction was stopped when α-hydroxyethylthiamine disappeared, and an estimate of relative yield of hydroxamic acid under varying conditions was obtained.

Title Reaction

α-Hydroxyethylthiamine HCl (*9*) [**4**, 3.8 g, 0.001 mole] and freshly recrystalized nitrosobenzene (*19*) [**5**, 3.3 g, 0.03 mole] were stirred under N_2 in 200 ml of DMF in a 3-neck RB flask equipped with a reflux condensor and dropping funnel. Triethylamine [3.0 g, 0.03 mol] in 5 ml of DMF was added dropwise in 2 min. The reaction mixture was heated at 70°C and stirred for 3 hr. The resulting brown solution was collected and combined with 200 ml H_2O, 200 ml sat NaCl solution, and 3 ml concd HCl, then extracted twice with 300 ml of H_2O, dried (Na_2SO_4), and evaporated *in vacuo* to give 3.5 g of a dark-brown oil. This oil was chromatographed on 100 g of silica gel eluting stepwise starting with $CHCl_3$ and ending with 5% MeOH/$CHCl_3$. Sixty 15-ml fractions were collected and combined on the basis of tlc analysis to give six fractions. Azoxybenzene (1.7 g, 59%) was obtained in essentially pure form from fraction 1 and was identified on the basis of comparing the ir and ms with those of authentic azoxybenzene. Comparative tlc analysis of the product with azoxybenzene in four solvent systems indicated a single compound chromatographing in an identical manner. Fraction 4 (0.065 g) gave a positive test for hydroxamic acids with $FeCl_3$ and was rechromatographed on 50 g silica gel by elution with Et_2O. Fractions were combined on the basis of tlc analysis with $FeCl_3$ visualization and evaporated to give 380 mg of a clear brown oil, which upon crystallization (C_6H_6) gave 200 mg (13%) of **7** as white needles. Mp and mmp [with authentic **7** (*13*)] 65.5–66.5°C; ir and ms identical with those of **7**. The product also displayed identical chromatographic characteristics with authentic **7**. Fraction 6 contained a strongly fluorescent spot as the major component, which chromatographed in a manner identical to that of thiochrome. Fractions 2, 3, and 5 consisted of complex mixtures of compounds in small quantities, and no attempt was made to characterize these side products.

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