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HYDROXAMIC ACID OXIDATION–PHARMACOLOGICAL CONSIDERATIONS

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Abstract—Both primary and secondary hydroxamic acids were found to be quite susceptible to oxidation by 2,6-dichlorophenolindophenol at physiological pH. Among the primary hydroxamates, substituted benzhydroxamic acids were more easily oxidized than aliphatic derivatives, resonance being an important factor determining relative rates. In the case of secondary hydroxamic acids, a wide range of oxidation rates was observed with steric hindrance and inductive effects being of prime concern. In that hydroxamates are of considerable interest, both as possible chemotherapeutic agents and as proximate carcinogens, we sought to correlate their relative rates of oxidation with carcinogenic potential. DNA binding studies showed that the rapidly oxidized *o*-hydroxybenzhydroxamic acid was not incorporated into DNA while *N*-hydroxy-2-acetylaminofluorene, which is less susceptible to oxidation, was incorporated rapidly.

The hydroxamic acid moiety is an essential component of a large number of compounds with a broad spectrum of biological and pharmacological activity. A diverse group of aliphatic and heterocyclic hydroxamic acids are synthesized by microbes [1, 2]. The major function of such compounds appears to be the acquisition and transport of environmental iron needed for growth. However, many hydroxamic acids exhibit antifungal [3], antibiotic [4] and even antitumor [5, 6] activity. Moreover, the exceptionally high affinity of hydroxamic acids for iron has prompted the evaluation of several compounds as potential iron-chelating drugs for use in the treatment of hemochromatosis, such as that which occurs in patients with β-thalassemia major [7, 8]. One of these, desferrioxamine, is now widely used for this purpose [9–13]. Several primary aromatic hydroxamic acids have been found to be potent inhibitors of the cyanide-insensitive alternate oxidase of plants [14, 15] and the terminal oxidase of the bloodstream forms of *Trypanosoma brucei brucei* [16–18].

Unique among the hydroxamic acids are the *N*-arylhydroxamates. These have been implicated as obligatory intermediates in the bioactivation of various carcinogenic aromatic amines [19, 20]. It has been proposed that the secondary hydroxamic acids so formed are either oxidized or esterified *in vivo* to form reactive intermediates which combine with macromolecules [19]. Presumably modification of the genetic material is responsible for the mutagenicity and carcinogenicity of the amines.

During the course of studying the effect of primary hydroxamates on electron transport in *T. b. brucei*, we were quite surprised to find that these compounds could directly reduce 2,6-dichlorophenolindophenol. Such a facile oxidation caused us immediate concern since we have been utilizing the hydroxamate moiety in designing both iron-chelating drugs [8] and trypanocidal agents. Accordingly, we decided to further evaluate the chemistry of this oxidation so as to gain insight into the potential hazards of using hydroxamic acids in chemotherapy. In this paper, we compare the rates of oxidation of a series of aromatic and aliphatic hydroxamates and discuss those factors which influence the rate.

MATERIALS AND METHODS

2,6-Dichlorophenolindophenol was obtained from Eastman Organic Chemicals (Rochester, NY). Desferrioxamine was supplied by the Ciba Pharmaceutical Co. (Summit, NJ). Other reagent grade chemicals were obtained from the Aldrich Chemical Co., Inc. (Cedar Knolls, NJ). DNA (type I, calf thymus) was purchased from the Sigma Chemical Co. (St. Louis, MO). Rhodotorulic acid was obtained from the supernatant fluid of cultures of *Rhodotorula pilimanae* (ATCC 46233) as described previously [8]. *N*-Hydroxy-2-acetylaminofluorene was the generous gift of Dr. M. C. Poirier of the NIH.

Salicyclic acid [7-^{14}C] (sp. act., 56 mCi/m-mole) and *N*-hydroxy-2-acetylaminofluorene[9-^{14}C] (sp. act., 19 mCi/m-mole) were obtained from New England Nuclear (Boston, MA) and ICN Pharmaceuticals, Inc. (Irvine, CA) respectively.

In addition to those compounds obtained commercially, a number of hydroxamic acids were prepared according to published procedures. Benzhydroxamic acid, *o*-chlorobenzhydroxamic acid, *m*-chlorobenzhydroxamic acid, *p*-chlorobenzhydroxamic acid. 2,4-dichlorobenzhydroxamic acid, *m*-bromobenzhydroxamic acid and *p*-nitrobenzhydroxamic acid were all prepared from the corresponding acid chlorides according to the method of Jones and Hurd [21]. *p*-Hydroxybenzhydroxamic acid was synthesized according to the procedure of Scott and Wood [22], as modified by Gale *et al.* [23]. *o*-Aminobenzhydroxamic acid was prepared from the corresponding methyl ester as described by Stolberg *et al.* [24]. Aceto-, hexano- and octanohydroxamic acids were synthesized according to the method of Fishbein *et al.* [25], glycylhydroxamic acid according to that of Safir and Williams [26] and histidylhydroxamic acid according to that of Cunningham *et al.* [27]. [^{14}C]-*o*-hydroxybenzhydroxamic acid (sp. act., 0.38 mCi/m-mole) was prepared according to the method of

Table 1. Secondary hydroxamates—Physical and analytical parameters

m-Chlorobenzhydroxamic acid derivative	M.p.* ( )	Formula	Analysis†	Yield (%)	Recrystallization solvent
N-Methyl-	56.5–58	$C_8H_8ClNO_2$	C,N,H	72.2	Cyclohexane-ether (1:1)
N-*n*-Hexyl-	49–50	$C_{14}H_{12}ClNO_2$	C,N,H	50.4	Petroleum ether (35–50°)
N-Cyclohexyl-	153–155	$C_{13}H_{16}ClNO_2$	C,N,H	74.5	Methanol
N-Benzyl-	150–151.5°	$C_{14}H_{12}ClNO_2$	C,N,H	97.0	Ether

*Melting points (uncorrected) were determined in capillary tubes.
†Where analyses are indicated only by symbols, the results were within ± 0.15%.

Urbanski [28], as modified by Gale *et al.* [23] and purified by high pressure liquid chromatography.

The secondary hydroxamic acids, *N*-methyl-*m*-chlorobenzhydroxamic acid, *N*-*n*-hexyl-*m*-chlorobenzhydroxamic acid, *N*-cyclohexyl-*m*-chlorobenzhydroxamic acid and *N*-benzyl-*m*-chlorobenzhydroxamic acid, were prepared from *m*-chlorobenzoylchloride and the appropriate *N*-alkylhydroxylamine according to the method of Jones and Hurd [21]. Except for *N*-methylhydroxylamine, which was obtained commercially, the *N*-alkylhydroxylamines were prepared as described by Borch *et al.* [29], the pH of the reaction mixture being maintained at 2.6 ± 0.3 by the addition of 2 N methanolic HCl so as to minimize formation of disubstituted hydroxylamines. The four secondary hydroxamic acids were previously unknown. Proton magnetic resonance spectra of the products were consistent with the structures proposed. Additional physical and analytical properties are presented in Table 1.

Rates of reduction of 2,6-dichlorophenolindophenol (DCIP) were determined spectrophotometrically (600 nm) using a Zeiss spectrophotometer (model PM6). Unless otherwise indicated, reactions were run at 22° in 0.09 M Tris–HCl buffer (pH 7.6) containing 10% methanol. Under these conditions, DCIP (0.056 mM) had an extinction coefficient of 1.79×10^9. Fresh solutions were prepared daily using doubly glass distilled water and spectral grade methanol as solvents. After introduction of the appropriate amount of hydroxamic acid, the absorbance of the solution was determined at 1- to 2-min intervals for the first half-hour and at longer intervals thereafter. Initial rates were determined and expressed relative to that of benzhydroxamic acid (BHA).

The interaction of oxidized ^{14}C-hydroxamic acids with DNA was studied at room temperature in 0.1 M Tris–HCl buffer (pH 7.6) containing 10% methanol. To incubation mixtures containing DNA (2 mg/ml) and either [^{14}C]-*N*-hydroxy-2-acetylaminofluorene (0.51 mM, 2.2×10^4 cpm/ml) or [^{14}C]-*o*-hydroxybenzhydroxamic acid (0.082 mM, 4.9×10^4 cpm/ml) were added either DCIP or $K_3Fe(CN)_6$ at a final concentration of 0.55 mM. At various times aliquots of the reaction mixtures were withdrawn and extracted (five to seven times) with water-saturated *n*-butanol until no radioactive material was taken up by the organic phase. Three vol. of ethanol was added, after which the precipitated DNA was collected by centrifugation, redissolved in water and finally reprecipitated via addition of 3 vol. of ethanol and 0.1 vol. of 2.5 M sodium acetate (pH 5.5). Incorporation of radiolabel was determined by counting a portion of the DNA in a liquid scintillation spectrometer (Packard, model 3375) and relating the activity to the absorbance (258 nm) of appropriate dilutions of an aqueous solution of the DNA.

RESULTS

In order to determine a convenient working range for studying the relative rates of oxidation of different hydroxamic acids, we examined the effects of pH and methanol on the rate of oxidation of *o*-hydroxybenzhydroxamic acid and benzhydroxamic acid in both phosphate and Tris buffer. The rate of reaction in phosphate buffer proved to be approximately half that observed in Tris. The latter rate varied with both the pH and methanol content of the buffer as shown by the representative data presented in Table 2. Based on these results, we chose to conduct our experiments at pH 7.6 in the presence of 10% methanol, conditions that allowed dissolution of a reasonable amount of the secondary hydroxamic acids.

Relative rates of reduction of DCIP by a series of primary aromatic hydroxamates are presented in Table 3. Compared to the rate of oxidation of BHA, introduction of an *o*-chloro group caused a 30 per cent decrease in the rate of reduction while a *p*-chloro substituent led to a 15 per cent enhancement. The effects of the two substituents are approximately additive, for 2,4-dichlorobenzhydroxamic acid was oxidized at almost the same rate as the parent compound. On the other hand, substitution at the

Table 2. Effect of pH and methanol on the rate of reduction of DCIP by hydroxamic acids (2 mM) in 0.9 M Tris–HCl buffer

Hydroxamic acid	pH	Methanol (%)	Rate (nmoles/hr)
Benz-	7.0	10	5.22
	7.6	10	2.36
	8.2	10	0.592
	8.8	10	0.269
o-Hydroxybenz-	7.6	0	12.5
	7.6	5	10.5
	7.6	10	8.81
	7.6	20	6.31

Table 3. Effect of various benzhydroxamic acids on the rate of reduction of DCIP

Hydroxamic acid	Concn (mM)	Rate (nmoles/hr)	Rate relative to BHA*
Benz-	4	3.54	1.00
	2	2.21	1.00
	1	1.44	1.00
o-Chlorobenz-	4	2.61	0.74
	2	1.67	0.76
	1	0.84	0.58
m-Chlorobenz-	4	7.69	2.17
	2	4.49	2.03
	1	2.30	1.60
p-Chlorobenz-	4	4.15	1.17
	2	2.81	1.27
	1	1.46	1.01
2,4-Dichlorobenz-	4	3.53	1.00
	2	2.24	1.01
	1	0.97	0.67
m-Bromobenz-	4	10.20	2.88
	2	5.64	2.55
	1	3.39	2.35
o-Hydroxybenz-	4	21.50	6.07
	2	8.81	3.90
	1	4.63	3.21
p-Hydroxybenz-	4	9.99	2.82
	2	3.65	1.65
	1	2.02	1.40
o-Aminobenz-	4	8.98	2.53
	2	3.42	1.54
	1	2.03	1.40
p-Nitrobenz-	4	4.80	1.36
	2	2.10	0.95
	1	1.04	0.73
N-Hydroxybenzene sulfonamide	1	154.0	104.0

*Benzhydroxamic acid.

meta position caused nearly a 2-fold acceleration of the rate in the case of the chloro compound and an even greater effect (2.6-fold) in that of *m*-bromobenzhydroxamic acid.

Also shown in Table 3 are the relative rates of the reaction upon incorporation of an *o*- or *p*-hydroxyl group or an *o*-amino group. In the case of *p*-hydroxybenzhydroxamic acid, a 2-fold acceleration of the rate was observed in comparison with the *p*-chloro derivative. Unlike the chloro analogue, however, *o*-hydroxy- and *o*-aminobenzhydroxamic acids were oxidized much more rapidly than the unsubstituted benzhydroxamic acid; these latter compounds showed a 3- and 2-fold enhancement of rate respectively. Furthermore, introduction of the electronegative nitro group into the para position had little effect on the rate of reduction. Also included in Table 3 is the rate at which *N*-hydroxybenzenesulfonamide was oxidized. Note that this was by far the most active compound, being oxidized 100 times faster than BHA.

As shown in Table 4, primary *n*-alkylhydroxamic acids react 60–80 per cent more slowly than benzhydroxamic acid, with the higher homologues being somewhat more reactive than acetohydroxamic acid. Introduction of an α-amino group caused a further diminution in the rate of reduction of DCIP. On the other hand, if the amino group was adjacent to the carbonyl of the hydroxamic acid moiety as in *N*-hydroxyurea, the rate of reduction of DCIP was enhanced approximately 30 times, relative to that of an α-aminohydroxamic acid, and was 1.5 times greater than BHA.

A comparison of primary and secondary hydroxamic acids was also undertaken. Since *m*-chlorobenzhydroxamic acid proved to be oxidized rapidly in this model system and since it has been shown to be a potent inhibitor of the cyanide-insensitive oxidases in both plants [14] and *T. b. brucei* [16], we prepared four *N*-alkyl derivatives of this compound. As shown in Table 5, *N*-methyl-*m*-chlorobenzhydroxamic acid reacted at a rate four times faster than benzhydroxamic acid and thus two times faster than *m*-chlorobenzhydroxamic acid. Increasing the length or bulk of the *N*-alkyl substituent, however, caused a diminution in the rate of oxidation of the hydroxamate. Thus, the *N*-*n*-hexyl and *N*-benzyl derivatives reacted only 60 per cent as fast as *m*-chlorobenzhydroxamic acid and the sterically hindered *N*-cyclohexyl derivative reacted only 10 per cent as fast. Moreover, *N*-hydroxy-2-acetylaminofluorene (N-OH-AAF), which is an *N*-arylhydroxamic acid, was seventeen times less reactive.

Also shown in Table 5 are the results obtained with

Table 4. Effect of various alkylhydroxamic acids on the rate of reduction of DCIP

Hydroxamic acid	Concn (mM)	Rate (nmoles/hr)	Rate relative toBHA*
Aceto-	4	1.01	0.29
	2	0.40	0.18
	1	0.29	0.20
Hexano-	4	1.37	0.39
	2	0.90	0.41
	1	0.66	0.46
Octano-	4	1.50	0.42
	2	0.73	0.33
	1	0.55	0.38
Glycyl-	4	0.072	0.020
Histidyl-	4	0.200	0.056
N-Hydroxyurea	4	6.51	1.84
	2	3.41	1.54
	1	2.00	1.39

*Benzhydroxamic acid.

desferrioxamine and rhodotorulic acid, two naturally occurring, multidentate secondary hydroxamic acids. While desferrioxamine reacted half as fast as BHA, rhodotorulic acid was only 5 per cent as reactive, As their iron chelates, both compounds were essentially unreactive.

Having thus determined that the oxidation of hydroxamic acids is a general reaction occurring under relatively mild conditions and proceeding over a wide range of rates, we investigated the possibility that products of the oxidation could be incorporated into DNA. The results are presented in Table 6. There was an increased binding of radiolabeled N-OH-AAF to DNA when oxidized with either DCIP or $K_3Fe(CN)_6$. The binding was approximately six times greater when the latter oxidant was used. Also of note is the fact that the reaction was essentially complete after incubating for 22 hr. On the other hand, when [^{14}C]-*o*-hydroxybenzhydroxamic acid was oxidized under the same conditions, no incorporation of radiolabel occurred using either oxidant.

DISCUSSION

The carcinogenicity of arylamines such as 2-aminofluorene is thought to arise as a result of their *in vivo* activation via *N*-acetylation and subsequent *N*-hydroxylation. The product, *N*-hydroxy-2-acetylaminofluorene, is considered to be a proximate carcinogen, that is, further biotransformation is required before carcinogenicity is exhibited. Miller [19] has postulated that the latter transformation involves either *O*-acylation or *O*-sulfation followed by decomposition of the resulting ester to a highly reactive arylnitrenium ion. More recently, however, evidence has been presented by a number of workers that N-OH-AAF readily undergoes one-electron oxidation to the nitroxide radical which can dismutate to yield 2-nitrosofluorene and *N*-acetoxy-2-acetylaminofluorene, both of which have been shown to be carcinogenic [30–33]. Using a variety of oxidants such as $K_3Fe(CN)_6$ [34], ceric sulfate-H_2SO_4 [35], silver oxide [36, 37] and hematin-cumene hydroperoxide [38], it has been possible to demonstrate the intermediacy of free radicals in the one-electron oxidation of hydroxamic acids via either electron spin resonance (e.s.r.) or ultraviolet spectrophotometry. In all cases, the major products isolated have been consistent with formation of an intermediate nitroxide radical. Moreover, Bartsch *et al.* [37, 39] have been able to demonstrate both e.s.r. signals and the two products noted above upon incubating N-OH-AAF with various peroxidases and H_2O_2. Thus, it has been suggested that one-electron oxidation of *N*-acylarylhydroxylamines may be an ultimate step in the *in vivo* activation of various aromatic amines and hydroxylamines.

From the data presented here, it is clear that an *N*-aryl substituent is not a prerequisite for facile oxidation of hydroxamic acids. Using DCIP, a relatively mild oxidant, we have shown that primary hydroxamates as well as *N*-alkylhydroxamic acids undergo similar reactions. Similarly, Boyland and Nery [40] studied the oxidation of primary hydroxamic acids by alkaline iodine and observed nitrous acid and *N,O*-diacylhydroxylamines as products. Furthermore, oxidation of primary hydroxamates in the presence of

Table 5. Effect of various secondary hydroxamic acids on the rate of reduction of DCIP

Hydroxamic acid	Concn (mM)	Rate (nmoles/hr)	Rate relative to BHA*
N-Methyl-*m*-chlorobenz-	4	14.60	4.14
	2	8.70	3.93
	1	5.33	3.70
N-*n*-Hexyl-*m*-chlorobenz-	1	1.73	1.20
N-Cyclohexyl-*m*-chlorobenz-	1	0.303	0.21
N-Benzyl-*m*-chlorobenz-	1	1.73	1.20
N-Hydroxy-2-acetyl-aminofluorene	0.5	0.157	
Desferrioxamine			
Desferrioxamine + Fe^{3+} (4 mM)	4	0.118	0.033
Rhodotorulic acid	4	0.189	0.053
Rhodotorulic acid + Fe^{3+} (4 mM)	4	0	0

*Benzhydroxamic acid.

Table 6. Incorporation of hydroxamic acids into DNA*

Hydroxamic acid	Time (Hr)	Oxidant None	DCIP	$K_3Fe(CN)_6$
N-hydroxy-2-acetyl-aminofluorene[9-^{14}C]	3	ND†	3.9	11.1
	22	3.6	5.6	16.1
	45	4.4	6.5	15.7
o-Hydrobenzhydroxamic acid[7-^{14}C]	3	0.052	0.045	0.046
	22	0.051	0.064	0·069
	45	0.020	0.010	0·010

†Not determined.
*Values are expressed as nmoles of hydroxamic acid incorporated per 100 A_{258}.

amines gave the corresponding amides [41], suggesting the formation of an electrophilic species (RCONO) as an intermediate. Secondary hydroxamates, on the other hand, were found to give nitroso dimers and carboxylic acids [42]. Although the presence of free radicals was not established in every case, they are presumed to be involved in all such oxidations (Fig. 1). No mechanistic studies have been published, however, in which the rate of oxidation has been correlated with the nature of the substituents R and R'.

The results presented in Tables 2–5 suggest several things about the mechanism of the oxidation. Adding methanol to the reaction mixture decreases the rate of oxidation of the hydroxamic acids. This suggests that free radicals play a role in the reaction, since primary aliphatic alcohols are known to act as radical scavengers [43, 44]. The effect of pH on the rate of DCIP reduction is probably due to the fact that DCIP is a stronger oxidant at lower pH [45]. Also of mechanistic import is the order of the reaction. Doubling either the concentration of DCIP (data not presented) or that of the hydroxamic acid caused an approximate doubling of the rate, as shown by the data in Tables 3–5. Thus, under these conditions, the reaction appears to be first order in both DCIP and hydroxamic acid.

In the series of benzhydroxamic acids (Table 3), a

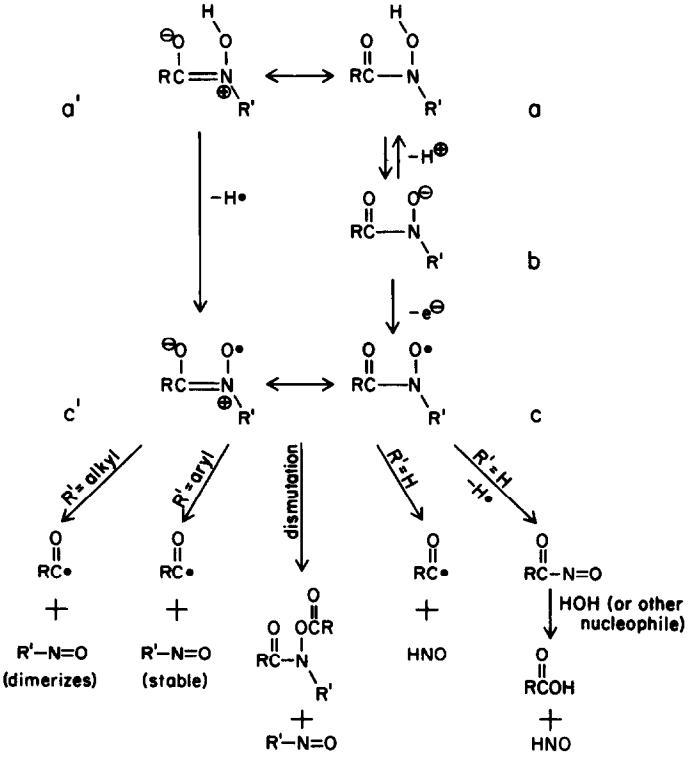

Fig. 1. Possible routes for the oxidative decomposition of hydroxamic acids.

3- to 4-fold difference in oxidation rates was observed upon introducing various meta and para substituents. At concentrations of 1 mM, the rates of the para-substituted benzhydroxamic acids were found to correlate with the Hammett substituent constants, suggesting that stabilization of species a' and c' (Fig. 1) is a rate-determining factor. Introduction of an ortho substituent capable of hydrogen bonding with the carbonyl oxygen seemed to increase the rate of oxidation significantly, further suggesting that increased electron density about the carbonyl oxygen may be an important mechanistic parameter. This is also indicated by the extremely rapid oxidation of *N*-hydroxybenzenesulfonamide.

Primary aliphatic hydroxamates tended to be less susceptible to oxidation than benzhydroxamic acids (Table 4). This is consistent with the inductive effects expected. The hydroxamates of α-amino acids, on the other hand, proved to be seven to ten times less reactive than the aliphatic hydroxamates. Because the pK_a values of the α-amino substituents in glycine and histidine are 9.60 and 9.17, respectively, one might expect the substituents in the corresponding hydroxamic acids to be protonated at pH 7.6, and thus to decrease the electron density on the carbonyl oxygen. This decrease in the rate of oxidation was not observed in the case of *o*-aminobenzhydroxamic acid. Since aniline is a weak base (pK_a 5.62), one would not expect the latter hydroxamic acid to be protonated at pH 7.6. The fact that its rate of reduction relative to BHA is slightly enhanced can be explained by consideration of the stabilization energy (approximately 3 kcal/mole) of this compound wherein the free electron pair on nitrogen is delocalized throughout both the aromatic ring and the carbonyl group. Attachment of the amino group directly to the hydroxamate moiety, as in *N*-hydroxyurea, caused a marked enhancement in the rate of oxidation relative to that of the α-amino hydroxamic acids. Here, too, delocalization of the free electron pair (stabilization energy ≃23 Kcal/mole) causes increased electron density on the carbonyl oxygen.

N-methyl-*m*-chlorobenzhydroxamic acid proved to be more reactive than the corresponding primary hydroxamate. This is in agreement with expectation as species *c'* of Fig. 1 should be stabilized by the inductive effect of the methyl group. Increasing the bulk of the *N*-alkyl substituent decreased the rate, however, presumably due to steric hindrance. The accessibility of the hydroxamate moiety also seems to play a role in the case of the naturally occurring di- and trihydroxamates, rhodotorulic acid and desferrioxamine. Both are derivatives of acetohydroxamic acid with desferrioxamine reacting slightly faster than its parent, perhaps due to the presence of multiple hydroxamate functionalities. Rhodotorulic acid, on the other hand, is less reactive even though both acyl substituents are acetyl groups. Thus, bulky substituents attached to nitrogen had a more profound effect upon the rate of reduction of DCIP than bulky acyl substituents. As expected, chelation of iron tended to protect the hydroxamate moiety from oxidation.

Because use of the hydroxamate moiety in chemotherapy is currently of considerable interest, it is of importance to determine whether the oxidizability of these compounds is reflected in an increased carcinogenic potential. It is impossible to answer this question fully at the present time. Of the two compounds currently employed as pharmacological agents, one, desferrioxamine, is oxidized quite slowly by DCIP while the other, *N*-hydroxyurea, is oxidized relatively rapidly. Desferrioxamine has been shown to be nonmutagenic (H. Marquardt, personal communication) while *N*-hydroxyurea is noncarcinogenic [46]. N-OH-AAF, a known carcinogen, also reduces DCIP very slowly so that the rate of oxidation alone may be a poor indicator of potential carcinogenicity. Furthermore, in the case of a series of *N*-hydroxy-*N*-acetylaminoarenes, it has been shown that the degree of carcinogenicity is inversely related to the rate of dismutation of the intermediate nitroxide radicals formed in their oxidation [37].

To investigate further the carcinogenic potential of the hydroxamates, we looked for binding of radiolabeled *o*-hydroxybenzhydroxamic acid and N-OH-AAF to DNA using either DCIP or $K_3Fe(CN)_6$ as oxidant. As shown in Table 6, no adduct was formed when *o*-hydroxybenzhydroxamic acid was oxidized with either oxidant. On the other hand, [^{14}C]-N-OH-AAF was incorporated into DNA upon oxidation with both oxidants. Clearly, there is a difference in the reactivity of the intermediate species derived from these two hydroxamic acids. We believe that the greater activity of N-OH-AAF is due to intercalation of either the compound itself or one of its reactive intermediates. Consistent with this proposal is the fact that the incorporation of radioactivity in the absence of oxidant was much greater in the case of N-OH-AAF. This mode of intercalation between polynuclear aromatic compounds and DNA has been suggested previously [47–49]. Intercalation of a reactive aryl intermediate would be expected to significantly enhance binding to DNA. Since *o*-hydroxybenzhydroxamic acid failed to react with DNA in spite of the fact that it is more easily oxidized than N-OH-AAF, it is reasonable to conclude that acylation of native DNA by oxidized hydroxamic acids is a minor reaction

Further studies of the potential carcinogenicity of both primary and secondary hydroxamic acids are warranted. This is true not only because of the pharmacological interest in these compounds, but also because of the relatively large number of hydroxamate precursors which are ingested by man.

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