Nucleophilic and Bifunctional Catalysis. Mechanism, Reactivity, and Transition-State Structure in the Hydrolysis of 2-Chloro-4-isopropylamino-6-cyclopropylamino-s-triazine by N-Hydroxysuccinimide and 1-Hydroxy-2-piperidone

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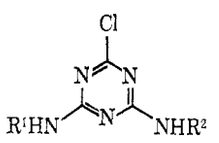
The hydrolysis in water at 70° of the herbicide 2-chloro-4-isopropylamino-6-cyclopropylamino-s-triazine (Cyprazine) is nucleophilically catalyzed by N-hydroxysuccinimide and 1-hydroxy-2-piperidone, which are models for natural plant resistance factors. N-Hydroxysuccinimide ($pK_a \sim 6$), which is more acidic than the transition state for its attack on the triazine nucleus ($pK_a \sim 7$), is 10-fold more nucleophilically reactive than its conjugate base, while 1-hydroxy-2-piperidone ($pK_a \sim 9$), which is less acidic than the transition state, is 25-fold less reactive than its conjugate base, in agreement with a general rule. Structural analysis of the transition states shows that the reduced acidity results from a bifunctional catalytic proton bridge in a reactant-like transition state. Application of the findings to the *in vivo* action of the corn-plant resistance factor demonstrates that the mechanism is adequate to describe the biological detoxification of the herbicide.

The ability of resistant plants to metabolize and detoxify 2-chlorobis(alkylamino)-s-triazine (**1**) herbi-



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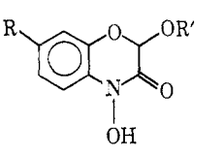
Compd	R^1	R^2
a, Simazine	C_2H_5	C_2H_5
b, Atrazine	i-C_3H_7	C_2H_5
c, Cyprazine	i-C_3H_7	

cides is now considered to be the basis for the selectivity of this class of herbicides rather than the degree of absorption of the herbicide by resistant plants or selective interference with certain biochemical processes by the herbicides in susceptible plants.[1-3] Three metabolic pathways for **1** are now known to exist, with the major pathway found in corn being dechlorination to give the 2-hydroxy analogs, which are relatively nonphytotoxic.[4-7]

The compound which is responsible for this metabolic inactivation reaction was isolated and identified as a benzoxazinone hydroxamic acid (**2**) which occurs as the glucoside.[8-12]

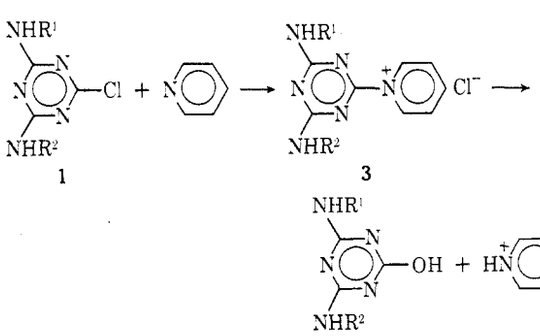
The dechlorination reaction caused by this cyclic hydroxamic acid has also been demonstrated to occur *in vitro*.[5,13] In resistant crops such as sorghum[6,14] and



2a, R = R′ = H
b, R = CH_3O; R′ = H
c, R = CH_3O; R′ = glucose

pea plants,[15,16] which do not contain the hydroxamic acid **2**, an N-dealkylation pathway has been shown to be responsible for the resistance to the chlorotriazine herbicides. Another metabolic pathway which has recently been reported is the formation of a glutathione conjugate of the triazine herbicide in the sorghum plant.[17]

Although evidence exists regarding the detoxification of the chlorotriazines (**1**) by the cyclic hydroxamic acids, **2a** and **2b**, studies concerning the mechanism of this process are very limited. Castelfranco and Brown[18] screened many nucleophilic agents for their ability to react with Simazine (**1a**) but found that only pyridine and hydroxylamine were effective. They suggested that nucleophilic attack occurred at carbon 2. The resulting intermediate **3** may undergo hydrolysis to give the 2-hydroxytriazine, **4** (eq 1). Tipton



1 **3** **4** (1)

(1) H. Gysin and E. Knuesli, *Advan. Pest Contr. Res.*, **3**, 289 (1960).
(2) M. L. Montgomery and V. H. Freed, *J. Agr. Food Chem.*, **12**, 11 (1964).
(3) R. H. Shimabukuro and H. R. Swanson, *J. Agr. Food Chem.*, **17**, 199 (1969).
(4) P. Castelfranco, C. P. Foy, and D. B. Deutch, *Weeds*, **9**, 580 (1961).
(5) R. H. Hamilton and D. E. Moreland, *Science*, **135**, 373 (1962).
(6) R. H. Shimabukuro, *Plant Physiol.*, **42**, 1269 (1927).
(7) W. Roth, *C. R. Acad. Sci.*, **245**, 942 (1957).
(8) A. V. Virtanen and P. K. Hietala, *Suom. Kemistilehti B*, **32**, 252 (1959).
(9) O. Wahlroos and A. I. Virtanen, *Acta Chem. Scand.*, **13**, 1609 (1959).
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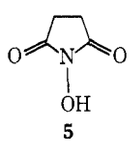
(12) E. Honkanen and A. I. Virtanen, *Acta Chem. Scand.*, **14**, 504 (1960).
(13) W. Roth and E. Knuesli, *Experientia*, **17**, 312 (1961).
(14) R. H. Shimabukuro, *Plant Physiol.*, **43**, 1925 (1968).
(15) R. H. Shimabukuro, R. E. Kadunce, and D. S. Frear, *J. Agr. Food Chem.*, **14**, 392 (1966).
(16) R. H. Shimabukuro, *J. Agr. Food Chem.*, **15**, 557 (1967).
(17) G. L. Lanoureux, R. H. Shimabukuro, R. H. Swanson, and D. S. Frear, *J. Agr. Food Chem.*, **18**, 81 (1970).
(18) P. Castelfranco and M. S. Brown, *Weeds*, **10**, 131 (1962).

and coworkers[19] have recently studied the reaction of Simazine with the hydroxamic acid **2b** and suggested that a molecular aggregate of **2b** may catalyze the reaction.

Since the reaction of the chlorotriazines **1** and the natural detoxifying agents **2** is slow[13] and **2** is unstable in hydroxylic solvents,[10,20,21] we decided to examine the reaction of 2-chloro-4-isopropylamino-6-cyclopropylamino-s-triazine (Cyprazine, **1c**) with more stable hydroxamic acids. The two cyclic hydroxamic acids which were selected as models for the natural system **2** were *N*-hydroxysuccinimide (**5**) and 1-hydroxy-2-piperidone (**6**). The absence of strong chromophores



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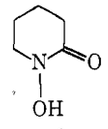

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in **5** and **6** permitted us to obtain detailed kinetic data by ultraviolet spectrophotometry.

Results

N-Hydroxysuccinimide-Catalyzed Hydrolysis of Cyprazine.—The ultraviolet absorption change with time during the reaction of Cyprazine (**1c**, 3.0×10^{-4} *M*) with *N*-hydroxysuccinimide (**5**) (20.0×10^{-4} *M*) at 70° in water containing 2% methanol is presented in Figure 1. The initial absence of isosbestic points shows that an intermediate (λ_{max} ~260 nm) accumulates during the first 2 hr. It then proceeds over a longer period to 2-hydroxy-4-isopropylamino-6-cyclopropylamino-s-triazine (**4**), to which the final spectrum corresponds exactly. If **4** arises from hydrolysis of an intermediate generated by reaction of Cyprazine and **5**, it should be isolable from their reaction in a nonaqueous medium. In fact, the product of the reaction in acetonitrile has λ_{max} 258 nm and a spectrum which resembles the 2-hr spectrum in Figure 1 (where the maximum at 258 nm can be seen as a shoulder) so that the isosbestic points observed after 2 hr at 228 and 255 nm corresponds to conversion of this compound to **4**.

Neither the intermediate compound **7** nor the product **4** nor *N*-hydroxysuccinimide (**5**) absorb at wavelengths beyond 280 nm, so that the decrease in absorbance at 285 or 290 nm gives a direct measure of the rate of disappearance of **1c**. Similarly, the increase in absorbance at 243 nm is specific for appearance of **4**. First-order plots of the data at 285 or 290 nm are linear with slopes independent of the initial concentration of reactant **1c**. The first-order rate constants, k_1' (Table I), are proportional to the concentration of *N*-hydroxysuccinimide (present in 6.7-fold to 27-fold excess) in both water and 0.1 *M* acetate buffer (pH 3.94) and are unaltered by the buffer at this pH. A plot of k_1' *vs.* concentration of **5** yields a second-order rate constant k_1 of 480 M^{-1} hr^{-1} at 70°.

First-order plots of the data at 243 nm show an initial lag time of 1–4 hr (depending on the concentration of **5**), followed by good first-order behavior. The first-order rate constant (Table I) is independent of the concentration of **1c**, **5**, and acetate buffer at pH 3.94, and is identical with that obtained in separate experiments for hydrolysis of the intermediate **7** to **4** and **5** under the same conditions.

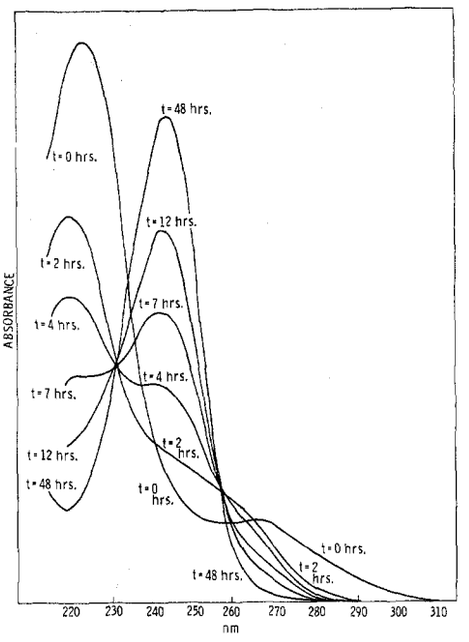


Figure 1.—Ultraviolet absorption as a function of time during the reaction of Cyprazine (**1c**, 3×10^{-4} *M*) with *N*-hydroxysuccinimide (**5**, 2×10^{-3} *M*) at 70° in water containing 2% methanol.

TABLE I

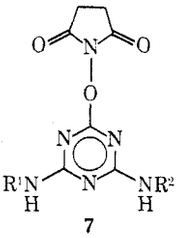
FIRST-ORDER RATE CONSTANTS IN THE REACTION OF CYPRAZINE (**1c**) WITH *N*-HYDROXYSUCCINIMIDE (**5**) IN WATER (1% METHANOL) AT 70°

10^4[**1c**], *M*	10^3[**5**], *M*	Buffer	k_1', hr^{-1}	k_2, hr^{-1}
1.50	1.00	None	0.444	0.130
1.50	2.00	None	0.820	
1.50	3.00	None	1.20	
3.0	4.00	None	2.01	0.126
1.50	2.00	Acetate[a]	0.94	0.128
1.50	3.00	Acetate[a]	1.50	
1.50	4.00	Acetate[a]	1.88	
1.50	0.00	Acetate[a]	0.023	

[a] Acetate buffer composed of 0.084 *M* acetic acid, 0.016 *M* sodium acetate (pH 3.9 at 70°), μ = 0.5 with added potassium chloride.

These data thus indicate a second-order reaction of Cyprazine with *N*-hydroxysuccinimide to form an intermediate **7**, which slowly hydrolyzes to **4** with regeneration of **5**. *N*-Hydroxysuccinimide is therefore a catalyst for the hydrolysis of Cyprazine.

Structure of the Intermediate 7.—The product **7** of the reaction of Cyprazine and *N*-hydroxysuccinimide



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(19) C. L. Tipton, R. R. Husted, and F. H. C. Tsao, *J. Agr. Food Chem.*, **19**, 484 (1971).

(20) J. B-Son Brendenberg, E. Honkanen, and A. I. Virtanen, *Acta Chem. Scand.*, **16**, 135 (1962).

(21) M. D. Corbett, Ph.D. Thesis, University of Kansas, 1970.

in acetonitrile is a monohydrochloride (silver nitrate, titration with 0.1 N NaOH) with a pK_a of 2.07 at 26° (*vs.* 1.6 for **1c**). It hydrolyzes to **4** and N-hydroxysuccinimide in water, as shown by thin layer chromatography. The nmr spectrum of the salt in $CDCl_3$ indicates the absence of a free OH group. These results and the analytical data are all consistent with the product **7** [R^1 = i-C_3H_7, R^2 = $CH(CH_2)_2$] from nucleophilic attack by the oxygen on **1c**.

Effect of pH.—The rate of attack of N-hydroxysuccinimide (**5**) on Cyprazine (**1c**) is independent of buffer concentration but varies with pH as shown in Table II. If the fall-off at higher pH is assumed to result

TABLE II

pH DEPENDENCE OF RATE CONSTANTS IN THE REACTION OF CYPRAZINE (**1c**) WITH N-HYDROXYSUCCINIMIDE (**5**) IN WATER (1% METHANOL) AT 70°

Buffer	[HA], M	[MA], M	pH	10^3 [5], M	k_1', hr^{-1}
CH_3CO_2H–	0.084	0.016	3.94	2.00	0.94
CH_3CO_2Na	0.084	0.016	3.94	0.00	0.023
	0.016	0.084	5.40	2.00	0.73
	0.016	0.084	5.40	0.00	0.002
KH_2PO_4–					
$Na_2HPO_4 \cdot 7H_2O$	0.050	0.050	6.8[a]	2.00	0.21

[a] Measured at 26°.

from ionization of N-hydroxysuccinimide to its conjugate base, then eq 2 should hold where k_{HA}' and k_A'

$$k_1' = k_{HA}'f_{HA} + k_A'(1 - f_{HA}) \quad (2)$$

are the pseudo-first-order rate constants for reaction of the acid and base forms, respectively, and f_{HA} is the fraction of **5** present as acid. Taking the pK_a of **5** as about 6 at 70° (it is 5.95 at 25°), eq 2 is obeyed for the three available points and gives k_{HA}' = 0.9 hr^{-1} and k_A' = 0.08 hr^{-1}. This remarkable result indicates that the neutral form of **5** is ten times more nucleophilic than its conjugate base toward Cyprazine.

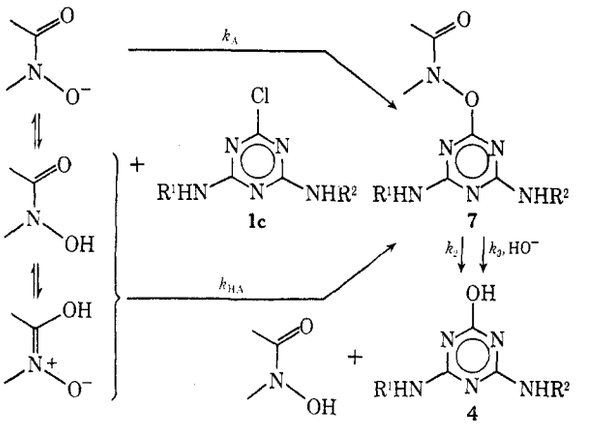
1-Hydroxy-2-piperidone Catalyzed Hydrolysis of Cyprazine.—The time variation of the ultraviolet spectrum in the course of the reaction of 1-hydroxy-2-piperidone (**6**) with Cyprazine is identical in character with that described for the N-hydroxysuccinimide reaction. Analysis of the data at 290 nm yields a rate constant k_1' for disappearance of **1c** at 70° in water of 0.01 hr^{-1} (0.002 M **6**, pH 4.4–6) while k_2 for conversion of the intermediate to **4** is 0.0027 hr^{-1}. At pH 10.0 in 0.1 M carbonate buffer, both rate constants greatly increased, k_1' to 0.25 hr^{-1}, k_2 to about 0.2 hr^{-1}. Thus, contrary to the situation with N-hydroxysuccinimide, nucleophilic attack by **6** on Cyprazine is base catalyzed. If this results from formation of the conjugate base of **6**, this species is about 25 times more reactive than neutral **6**. The increase in k_2 at high pH is indicative of base catalysis in the hydrolysis of **7**.

Activation Parameters.—A rough determination of the rate of the N-hydroxysuccinimide reaction with Cyprazine at 40° yields a rate constant $k_1 \cong 95$ M^{-1} hr^{-1}. Together with the value of 480 M^{-1} hr^{-1} at 70°, this gives the approximate activation parameters $\Delta G_{343}^* = 2.16$ kcal/mol, $\Delta H^* = 11$ kcal/mol, and $\Delta S^* = -31$ gibbs/mol. From these, we estimate $k_1 \cong 37$ M^{-1} hr^{-1} at 25°.

Discussion

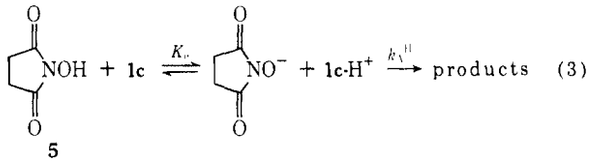
Mechanism.—All data given above are consistent with the mechanism of Scheme I for hydroxamic acid

SCHEME I



catalyzed hydrolysis of Cyprazine. Both the anion and the neutral form of the hydroxamic acid are capable of displacing chloride from the chlorotriazine nucleus to give intermediate **7**. The latter then undergoes acid-, neutral, and base-catalyzed hydrolysis; the mechanistic details of this process will be treated in another report.

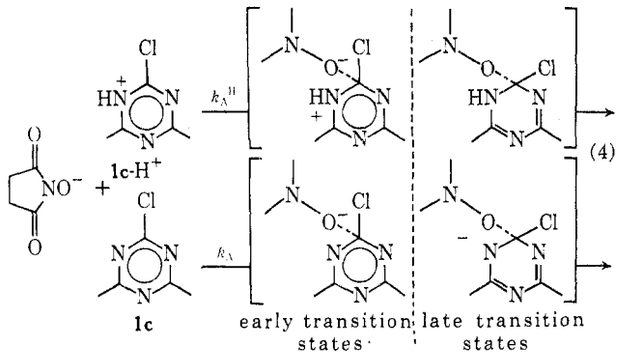
Relative Reactivities of Neutral and Anionic Forms.—One of the notable features of these findings is that the anion of 1-hydroxy-2-piperidone is about 25 times more nucleophilically reactive than its neutral form toward Cyprazine, while the neutral form of N-hydroxysuccinimide is ten times more reactive than its anion. A conventional explanation for the latter kind of observation (greater nucleophilic reactivity of the conjugate acid than the nucleophile itself) is that the rate constant k_{HA} does not reflect direct attack of N-hydroxysuccinimide on **1c** but rather an initial exchange of a proton between the reactants, followed by attack of the anion of N-hydroxysuccinimide on **1c**-H^+ (eq 3). Then the rate



(3)

constant $k_{HA} = K_e k_A^H$ and since $K_e = K_a^5/K_a^{1c\text{-}H}$, $k_{HA} = K_a^5 k_A^H/K_a^{1c\text{-}H}$. It is possible in this case for k_{HA} to exceed k_A, the rate constant for attack of the anion of **5** directly on **1c**, but there is no *a priori* way to tell whether it will or not. It is likely that $k_A^H > k_A$, since protonation of **1c** should activate it toward nucleophilic attack, but the question is whether this activation is sufficient to overcome the effect of the initial unfavorable proton transfer from **5** ($pK_a \sim 6$) to **1c** ($pK_a^{1c\text{-}H} \sim 1.6$). In other words, $k_{HA} \cong 10^{-4.4} k_A^H$ on this model, and unless $k_A^H > 10^{4.4} k_A$, then the conventional wisdom must fail to explain why $k_{HA} > k_A$. In fact, the relative magnitudes of k_A^H and k_A depend on the structure of the transition

state for nucleophilic attack (eq 4). Early transition states resemble the reactants **1c** and **1c**-H^+ strongly, so



that transition-state free-energy differences will be cancelled by reactant-state differences and $k_A^H \sim k_A$. Late transition states greatly favor protonation of the ring nitrogen, so that now k_A^H will tend to become larger than k_A. Transition states for nucleophilic attack on substrates like **1c** are often thought, from the Hammond postulate for example, to have product-like structures because of the instability of the nucleophilic adduct (tetrahedral intermediate, Meisenheimer complex, etc.) relative to reactants. Thus the conventional wisdom may succeed in cases where this prediction is accurate.

However, it is important to notice that it is very difficult to establish or even to test mechanisms of the form of eq 3 for processes of this type. One is attempting to infer the route by which molecules leave the reactant state and travel to the transition state from data which in general refer only to free-energy differences between the initial and final states. It is therefore more desirable to formulate the problem of relative *phenomenological reactivities* of nucleophiles and their conjugate acids (*i.e.*, relative magnitudes of k_A and k_{HA}) in terms which are independent of the route by which reactants reach the transition state. This is particularly true because there are other reasons for greater reactivity of nucleophile conjugate acids than the conventional one reviewed above. We find that the concept of transition-state acidities[22] offers a convenient method for treating the problem.

Scheme II shows the thermodynamic cycle from which the transition-state acidities can be deduced.[22] As in other thermodynamic cycles, the free-energy changes are state functions and no mechanistic knowledge of the route from one state to another is implied by the scheme. In Scheme II, transition state **8** contains one more proton than transition state **9** and the two are therefore a conjugate acid–base pair, connected by the ionization constant K_a^*. The exact position of the proton in **8** is not specified but is discussed below. The ionization constant K_a^* can be calculated from experimental data because Scheme II constitutes a closed thermodynamic cycle; thus $K_a^* = K_a k_A/k_{HA}$, or $pK_a^* = pK_a - \log k_A/k_{HA}$. For *N*-hydroxysuccinimide (R_2 = O in Scheme II), $pK_a \sim 6$ and $k_A/k_{HA} \sim 0.1$ so that $pK_a^* \sim 7$ for **8b**. For 1-hydroxy-2-piperidone ($R_2 = H_2$ in Scheme II), $pK_a \sim 9$ and $k_A/k_{HA} \sim 25$ so that $pK_a^* = 7.6$ for **8a**.

(22) J. L. Kurz, *Accounts Chem. Res.*, **5**, 1 (1972).

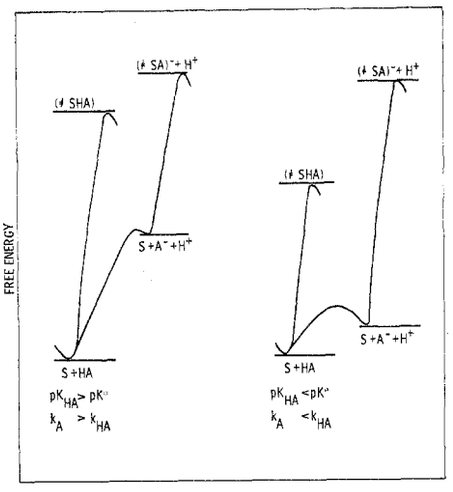
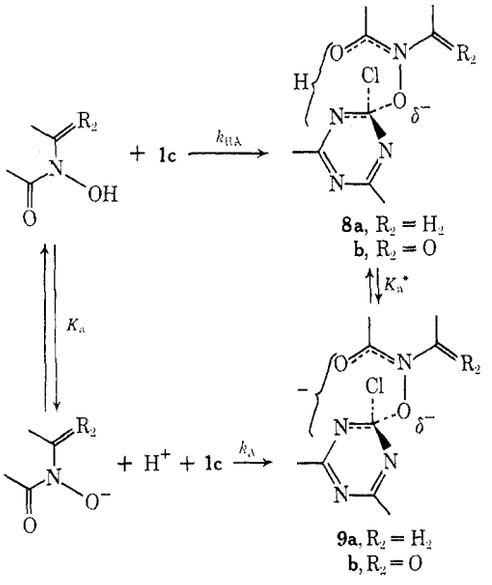


Figure 2.—Free-energy diagram illustrating that nucleophiles which are more acidic than the transition states for their nucelophilic attack ($pK_a < pK_a^*$) are also more reactive than their conjugate bases ($k_{HA} > k_A$) and vice versa.

SCHEME II



The transition states for attack by both neutral nucleophiles are thus about equally acidic, the pK_a^*'s being 7.6 for **8a** and 7 for **8b**. Now *N*-hydroxysuccinimide, with a pK_a of about 6, is a somewhat stronger acid than these transition states, while 1-hydroxy-2-piperidone, with a pK_a of about 9, is a considerably weaker acid than the transition states. As Figure 2 shows schematically, this situation is covered by the following general rule.

Whenever a nucleophile is more acidic than the transition state for its nucleophilic attack, it will be more nucleophilically reactive than its conjugate base; if a nucleophile is less acidic than the transition state for its nucleophilic attack, it will be less reactive nucleophilically than its conjugate base.

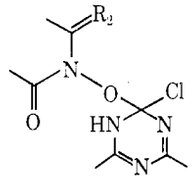
As can be seen from Figure 2, ionization of a relatively weak reactant acid produces a conjugate base closer to its corresponding transition state in free energy and thus more reactive than the conjugate acid. A relatively strong acid, on the other hand, ionizes to a species more

distant in free energy from its transition state and therefore less reactive than its conjugate acid.

Usually transition states for nucleophilic attack by ionizable nucleophiles are stronger acids than the free nucleophile because the proton remains bound to the nucleophilic atom in the transition state. The latter is becoming more positive and thus the proton is more acidic. Then the nucleophilic reactivity of the anion always exceeds that of its conjugate base. Here the opposite is true in the case of *N*-hydroxysuccinimide, for which reasons are considered below.

The correctness of the rule we state above is obvious from the method of calculation of K_a^* (*i.e.*, $k_{HA}/k_A = K_a^*/K_a^{HA}$ so that $k_{HA} > k_A$ only when $K_a^* > K_a^{HA}$) but it is by no means a restatement of this equation or a mere tautology. K_a^* is a good thermodynamic property of the transition state and the molecular structure of the transition state determines its value; thus some transition-state structures can lead to $k_{HA} > k_A$ while others cannot.

What is the factor (or factors) which makes transition state **8** so weakly acidic? One explanation, corresponding to the conventional picture of eq 3 and 4, is that **8** has a structure approaching **11**, which might have a pK_a (for loss of the proton from the ring NH) as large[23] as 12. On this model, the acidity constant of **8**, K_a^*, will vary from $10^{-1.6}$ (when it exactly resembles reactant **1c**-H^+) to 10^{-12} (when it exactly resembles **11**).



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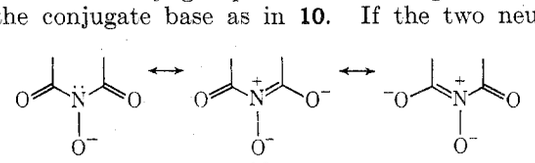
Thus $K_a^* = (10^{-1.6})^{1-x}\,(10^{-12})^x$ where x is an index of transition state structure which varies from 0 (reactant like) to 1 (product like). In fact, if the O–C bond order from nucleophile to ring is a good measure of transition-state structure, as it should be, then the negative charge δ on the nucleophilic oxygen of **8** will vary from $-\delta = -1$ (reactant-like) to $-\delta = 0$ (product-like), so that $\delta = 1 - x$, $1 - \delta = x$. Therefore $K_a^* = (10^{-1.6})^{\delta} \cdot (10^{-12})^{1-\delta}$ and, since $pK_a^* = 7$ for **8a** and 7.6 for **8b**, we obtain $-\delta = -0.5$ (**8a**), $-\delta = -0.4$ (**8b**) *if the conventional model of eq 3–4 is correct.*

Now the conventional model can be tested because the charges δ in **8** and δ' in **9** (Scheme II) of the nucleophilic oxygens can also be inferred in the following independent way. *N*-Hydroxysuccinimide is 10^3 times more acidic than 1-hydroxy-2-piperidone because the additional carbonyl group stabilizes the negative charge in the conjugate base as in **10**. If the two neutral nucleophiles were converted into a transition state with a full negative charge on the nucleophilic oyxgen, then the same stabilizing effects should come into play and *N*-hydroxysuccinimide should react 10^3 times faster. Actually the ratio k_{HA} (**5**)$/k_{HA}$ (**6**) is about 90. The usual linear free energy concepts thus suggest that $\delta = \log 90/\log 10^3 \sim 0.7$, *i.e.*, that transition state **8** has a charge $-\delta$ of -0.7 on its nucleophilic oxygen. A similar argument indicates that, if the anionic nucleophiles were converted to a transition state with *zero* negative charge on the nucleophilic oxygen, then the less stable 1-hydroxy-2-piperidone anion should react 10^3 times faster. In fact, it reacts only three times faster, meaning that the charge has decreased only a fraction $\log 3/\log 10^3$ or about 0.2 of the way from one to zero. That is, $-\delta' = -(1 - 0.2) = -0.8$ in **9**, about the same as $-\delta = -0.7$ in **8**. The large quantity of charge still on this oxygen shows that the bond to the triazine ring is only slightly (20–30%) formed in both **8** and **9**.



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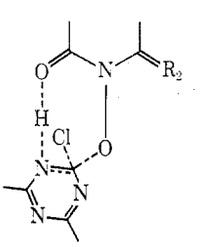
Returning now to the conventional model discussed above, we notice that only if $-\delta$ were as small as -0.4 in **8b** (transition state C–O bond about 60% formed) would the NH bond be sufficiently lacking in acidity in **8b** to permit $k_{HA} > k_A$. Since the independent measure of δ just made shows that $-\delta$ is actually about -0.7 in **8** (transition state C–O bond only 30% formed), the conventional model does not account for the findings.

We conclude that the transition state for nucleophilic attack on **1c** is an early one, so early that *N*-hydroxysuccinimide would react more slowly than its conjugate base, *even by prior proton transfer*, unless some special interaction were present to stabilize the proton of **8** and render it less acidic.[24] The most reasonable interaction is a bridging of the proton between O and N as in **12**, a form of bifunctional catalysis in which one function performs a protolytic role, the other a nucleophilic role.



Application to the *in Vivo* Action of the Natural Resistance Factor.—The natural detoxifying agent present in corn, **2b**, has a pK_a of 6.4 at room temperature. Assuming that the transition-state acidities are related to the hydroxamic acid acidities by a linear free energy equation, and knowing that the transition states for the reaction of *N*-hydroxysuccinimide ($pK_a = 6$) and 1-hydroxy-2-piperidone ($pK_a = 9$) have pK_a's of 7 and 7.6, respectively, we estimate that $pK_a^* = 7.1$ for **2b**. This is turn tells us that (since $pK_a < pK_a^*$) the neutral form of **2b** should be more reactive than its anion; in fact $k_{HA}/k_A \sim 5$ $(= K_a/K_a^*)$. From the separate linear free energy relations for k_A *vs.* K_a and k_{HA} *vs.* K_a, we obtain that k_{HA} for **2b** is about 1.8-fold smaller than k_{HA} for *N*-hydroxysuccinimide while k_A is about 1.1-fold larger than k_A for *N*-hydroxysuccini-

(23) This estimate is based on analogies tabulated by G. Kortüm, W. Vogel, and K. Andrussow, "Dissociation Constants of Organic Acids in Aqueous Solution," Butterworths, London, 1961, by A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen, London, 1962, and by G. Yagil, *Tetrahedron*, **23**, 2855 (1967), and should be regarded as a maximum value. Note that a smaller, more realistic value will intensify the argument offered below.

(24) This concept has some affinities with the ideas of J. Hine, "Physical Organic Chemistry," McGraw-Hill, New York, N. Y., 1962, p 111, and of W. P. Jencks, *Chem. Rev.*, **72**, 705 (1972).

mide. The temperature dependence of k_{HA} for *N*-hydroxysuccinimide gives $k_{HA} \sim 37\ M^{-1}\ hr^{-1}$ at 25°, approximating *in vivo* conditions and the correction factor of 1.8 for **2b** yields $k_{HA} \sim 21\ M^{-1}\ hr^{-1}$ for the natural resistance factor *in vivo*. The ratio $k_{HA}/k_A \sim 5$ then provides $k_A \sim 4\ M^{-1}\ hr^{-1}$. Thus the effective *in vivo* rate constant for action of the corn plant resistance factor against Cyprazine varies from $\sim 21\ M^{-1}\ hr^{-1}$ below pH 5.4 to about $4\ M^{-1}\ hr^{-1}$ above pH 7.4. Small's summary[25] of pH measurements on tissues of *Zea mays* indicates a range from 5.19 to 5.68 in normal plants. The effective rate constant thus varies only from about 21 to about $18\ M^{-1}\ hr^{-1}$, giving an average around $20\ M^{-1}\ hr^{-1}$.

To find the time required for detoxification by this mechanism we assume that the resistance factor is in large excess over intracellular Cyprazine. The loss of Cyprazine will proceed with a first-order rate constant $k_{eff} = 20\ M^{-1}\ hr^{-1}$ (concentration of **2b**, *M*). Various measurements of tissue levels of corn plant resistance factors indicate an average concentration of approximately $5 \times 10^{-3}\ M$, which gives $k_{eff} = 0.10\ hr^{-1}$. This corresponds to a half-life of about 7 hr for intracellular Cyprazine, or complete (99%) detoxification in about 2–3 days.

The fact that the natural resistance factor is more acidic ($pK_a = 6.4$) than its nucleophilic transition state ($pK_a^* = 7.1$) is of some significance since it is responsible for the high efficiency of detoxification even under the relatively acidic conditions prevailing in the corn tissue.

Experimental Section

Materials.—Cyprazine (**1c**) supplied by Gulf Research and Development Co. was recrystallized twice from toluene followed by two recrystallizations from chloroform–petroleum ether (bp 60–68°), mp 161–164° (lit.[26] mp 167–168).

N-Hydroxysuccinimide (**5**) (Aldrich Chemical Co.) was purified by repeated crystallization from methanol–ethyl acetate to correct elemental analysis. The pK_a was determined to be 5.95 by titration with 0.1 *N* NaOH.

1-Hydroxy-2-piperidone (**6**) was prepared according to the method of Panizzi, *et al.*,[27] by reaction of *N*-hydroxybenzenesulfonamide with cyclopentanone at 0°. It was purified by repeated sublimation at 50–55° (0.1 mm), mp 55°, $pK_a = 9.15$ (determined spectrophotometrically at 26°).

Basic alumina, chromatographic grade (E. Merck A. G., Darmstadt) and reagent grade chemicals were utilized.

Reaction of Cyprazine (1c) with *N*-Hydroxysuccinimide (5) in Acetonitrile.—Cyprazine (**1c**, 9.79 g, 0.043 mol) and *N*-hydroxysuccinimide (**5**, 4.95 g, 0.043 mol) were placed in a 500-ml erlenmeyer flask and enough acetonitrile was added to effect solution. The flask was maintained at 70° for 3 days, after which the solvent was removed *in vacuo* and the residue was dissolved in chloroform. The solution was warmed and ether was added to the cloud point. Upon cooling an oily substance separated as the lower layer. The addition of ethyl acetate to the oil caused the precipitation of the desired substance. The precipitate was collected and washed with ether–ethyl acetate. The chloroform–ether solution from which the oily substance separated was evaporated to dryness and the residue was washed with ethyl acetate and combined with the solid obtained previously. The yield of the crude product as the hydrochloride salt was 9.80 g (66.5%), mp 191–194° dec. Purification of the salt by recrystallization was not successful; thus it was converted to the free base by chromatography on basic alumina. The crude salt (6.0 g) was eluted with ethyl acetate from 210 g of basic alumina to give 3.8 g (71%) of a residue which was recrystallized from ethyl acetate–ether: mp 179–181°; λ_{max} 220 nm (acetate buffer, pH 4.0, ϵ 3.8×10^4), 217 (0.01 *N* $HClO_4$, 2.9×10^4), 257 (0.01 *N* $HClO_4$, 7.9×10^3); nmr ($CDCl_3$) δ 2.82 (s, 4, O=CCH_2CH_2C=O); ir (KBr) 1745 cm^{-1} (C=O); *m/e* 3.06.3.

Anal. Calcd for $C_{12}H_{18}N_6O_3$: C, 50.96; H, 5.91; N, 27.49. Found: C, 50.73; H, 5.93; N, 27.18.

Kinetic Procedure.—An aliquot of the hydroxamic acid solution in water (2–8 ml, 0.05 *M*) was placed into each of two 100-ml volumetric flasks and diluted to about 95 ml with water. When the reaction was performed in buffers the calculated amounts of buffer components were added together with the calculated amount of KCl to maintain the ionic strength at 0.5 *M* and the contents of the flasks were then diluted to about 95 ml with water. The flasks were immersed in a constant-temperature bath at 70.00 ± 0.5°. After thermal equilibration an aliquot (1 or 2 ml) of a stock solution of chlorotriazine ($1.5 \times 10^{-2}\ M$ in MeOH) was added to one flask and the same volume of methanol was added to the other flask. The flasks were then filled to the mark with water maintained at the same temperature, shaken, and returned to the constant-temperature bath. Zero time samples (5 ml) were withdrawn immediately and sampling was continued at appropriate time intervals. The samples were acidified by addition of 0.5–2.0 ml of 1 *N* $HClO_4$ or H_2SO_4 and diluted to 50 ml with water. The spectra or absorbances at a fixed wavelength were obtained on a Cary 14 spectrophotometer against the control sample solutions which were obtained in the identical manner from the control reaction mixture. Absorbance was measured using 10-cm cells at either 285 or 290 nm, and in 2- or 1-cm cells at 243 nm.

Thin Layer Chromatography.—Samples were spotted on precoated silica gel F 254 plates (Brinkmann Instruments) and developed to a distance of 7 cm using two solvent systems: (1) *n*-butyl alcohol–acetic acid–water (5:1:4) upper layer; and (2) isopropyl alcohol–ammonia–water (80:5:15) upper layer.

The fact that the salt hydrolyzes in water to 2-hydroxytriazine **4** and regenerates *N*-hydroxysuccinimide (**5**) was confirmed by tlc. The salt solution in water was allowed to stand for several days at room temperature, spotted on silica plates, and developed with two different solvent systems, 1 and 2. The R_f's observed with solvent systems 1 and 2, respectively, were 0.74 (**7**), 0.60 (**4**), and 0.41 (**5**) and 0.44 (**7**), 0.56 (**4**), and 0.11 (**5**). The reaction mixture gave a faint red-purple color with $FeCl_3$, indicating the possible presence of **5**.

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Registry No.—**1c**, 22936-86-3; **5**, 6066-82-6; **7**, 42449-58-1; **7** HCl, 42449-59-2.

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(26) R. P. Neighbors and L. V. Phillips, South African Patent 6,802,975 (Oct 21, 1968); *Chem. Abstr.*, **71**, 39013x (1969).

(27) L. Panizzi, G. DiMaio, P. A. Tardella, and L. d'Abbiero, *Ric. Sci.*, **1**, IIA, 312 (1961); *Chem. Abstr.*, **57**, 9658 (1962).