Detection of *N*-Acetyl-*p*-benzoquinone Imine Produced during the Hydrolysis of the Model Phenacetin Metabolite *N*-(Pivaloyloxy)phenacetin

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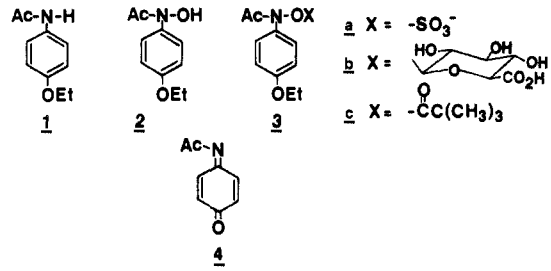
N-Acetyl-*p*-benzoquinone imine (**4**) can be detected by UV spectrophotometry and HPLC during the hydrolysis of *N*-(pivaloyloxy)phenacetin (**3c**). This material serves as a model for the sulfate and glucuronide conjugates (**3a** and **3b**) of *N*-hydroxyphenacetin (**2**). Direct detection of **4** during the hydrolysis of **3a** and **3b** is precluded by the extreme instability of **3a** and the very slow hydrolysis of **3b**. Our data show that >90% of the hydrolysis of **3c** proceeds through the intermediate **4** at all pH values in the range 1.0–8.0. All our results indicate that **4** is produced through a nitrenium ion mechanism. Many of the differences in the hydrolysis reactions of **3b** and **3c** can be explained in terms of a mechanism involving tight and solvent-separated nitrenium ion pairs. Other differences may be due to a homolysis pathway, which is more important for the material with the poorer leaving group (**3b**).

It has been shown that liver microsomes of several mammalian species convert phenacetin (**1**) into *N*-hydroxyphenacetin (**2**),[1] and *N*-hydroxyphenacetin has been found in the urine of rats dosed with phenacetin.[2] In vitro and in vivo experiments indicate that **2** is conjugated with sulfate and glucuronic acid by liver enzyme systems to form the reactive metabolites **3a** and **3b**.[3] These species decompose in aqueous media to yield an arylating intermediate.[4] Evidence based on product studies and kinetic analysis has indicated that this intermediate is the liver toxin *N*-acetyl-*p*-benzoquinone imine (**4**), but **4** has not been directly detected as a breakdown product of these materials.[4] The sulfate ester **3a** has proven to be too unstable to isolate,[4] and the glucuronide **3b** decomposes too slowly to allow detectable quantities of the reactive intermediate **4** to build up during its hydrolysis.[4]



Our work with sulfuric and pivalic acid ester derivatives of the *N*-hydroxyacetanilides has shown that the rate of decomposition of these species in aqueous media is strongly dependent on the pK_a of the acid.[5] The pivalic acid derivatives are always considerably more stable than the corresponding sulfuric acid esters, but would be much more reactive than glucuronides. Accordingly, we reasoned that the model phenacetin metabolite *N*-(pivaloyloxy)phenacetin (**3c**) should be stable enough to be isolated, but also reactive enough that **4** may be directly detected as a product of its decomposition in aqueous solution.

The results of our experiments on the hydrolysis reactions of **3c**, which support our hypothesis, are reported herein.

Results and Discussion

N-(Pivaloyloxy)phenacetin (**3c**) can be synthesized from the reaction of **2** with pivaloyl chloride in dry ether. The crude product is ca. 90–95% pure according to ^1H NMR and HPLC analysis (see Experimental Section). Attempts to purify **3c** have failed, since the compound is quite labile and decomposes in organic solvents by rearrangment to two isomeric ring-substituted (pivaloyloxy)phenacetins.[6] All spectral data obtained for **3c** are consistent with the assigned structure and are comparable to data we have obtained previously for other *N*-(pivaloyloxy)acetanilides.[5c,7] Freshly prepared samples of **3c** contain only small quantities of the rearrangement products. Since these impurities are unreactive in aqueous solution at moderate temperatures, they do not interfere with our experiments.

Figure 1A shows repetitive wavelength scans of UV absorbance taken during the hydrolysis of **3c** in 0.01 M acetate buffer (μ = 0.50 M (KCl)), pH 4.60, at 25 °C. It is instructive to examine absorbance vs. time behavior at two wavelengths: 266.5 and 245 nm. At 266.5 nm the absorbance initially rises rapidly, reaching a maximum ca. 60 s into the reaction, and then decreases at a slower rate. Absorbance vs. time data can be fit[8] very well to eq 1, the rate equation for two consecutive first-order processes.

$$A_t = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_\infty \quad (1)$$

The two rate constants obtained from this treatment, k_0 and k', are $(2.63 \pm 0.05) \times 10^{-2}$ s^{-1} and $(3.70 \pm 0.02) \times 10^{-3}$ s^{-1}, respectively. At 245 nm the two processes corresponding to k_0 and k' can still be observed; however, at this wavelength a third, slower event can also be observed. Absorbance vs. time data obtained at 245 nm are best fit[8]

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(6) These have been tentatively identified as 2-(pivaloyloxy)-phenacetin and 3-(pivaloyloxy)phenacetin. Analogous materials have been isolated from the reactions of *N*-(pivaloyloxy)-4-methylacetanilide.[5c]

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Table I. Pseudo-First-Order Rate Constants for the Decomposition of 3c at 25 °C

buffer[a]	pH[b]	10^2k_0, s^{-1}	k', s^{-1}	k'', s^{-1}
HCl	1.00	2.71 ± 0.07	c	(6.06 ± 0.10) × 10^{-3}
HCl	2.00	2.52 ± 0.05	c	(9.96 ± 0.07) × 10^{-4}
HCl	3.02	2.05 ± 0.07	(1.55 ± 0.16) × 10^{-1}	(4.76 ± 0.02) × 10^{-4}
KOAc/HOAc	3.71	2.46 ± 0.12	(2.46 ± 0.12) × 10^{-2}	(4.71 ± 0.02) × 10^{-4}
KOAc/HOAc	4.60	2.63 ± 0.05	(3.70 ± 0.02) × 10^{-3}	(6.42 ± 0.02) × 10^{-4}
KOAc/HOAc	5.64	2.24 ± 0.01	(3.94 ± 0.01) × 10^{-4}	(2.61 ± 0.17) × 10^{-3}
K_2HPO_4/KH_2PO_4	5.75	2.26 ± 0.01	(3.03 ± 0.01) × 10^{-4}	(2.87 ± 0.07) × 10^{-3}
K_2HPO_4/KH_2PO_4	6.77	2.19 ± 0.02	(5.12 ± 0.10) × 10^{-5}	c
K_2HPO_4/KH_2PO_4	7.55	2.14 ± 0.01	(2.88 ± 0.09) × 10^{-5}	c

[a] Ionic strength 0.50 M (KCl). Acetate and phosphate buffers were 0.01 M in total buffer. [b] ±0.02 at 25 °C. [c] Too fast to measure by conventional methods.

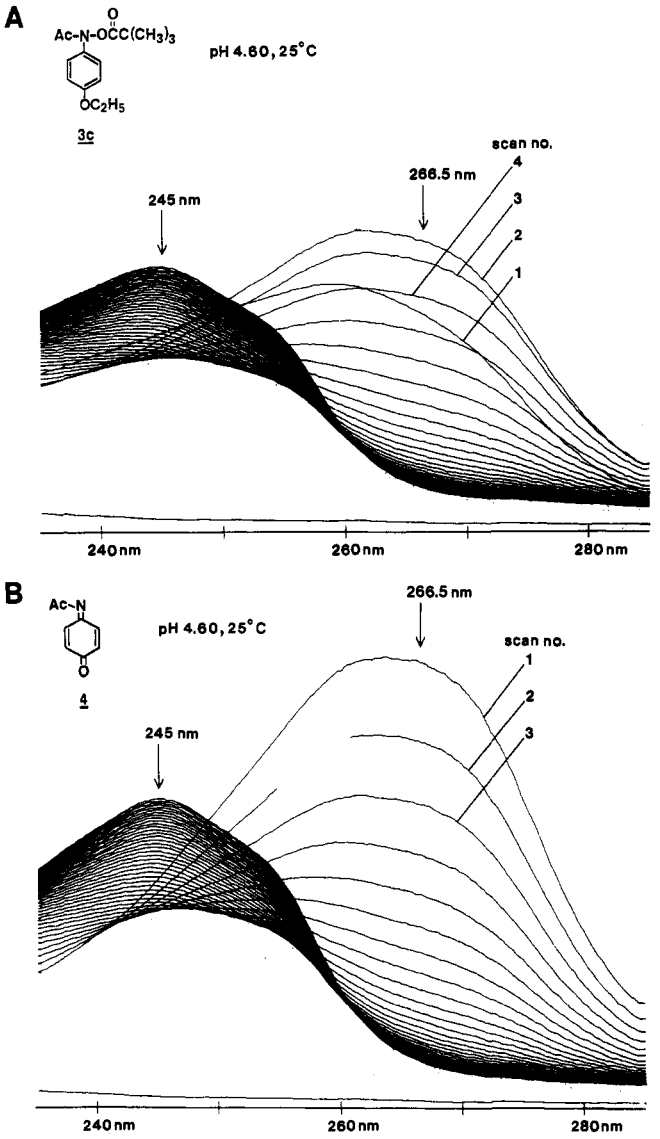

Figure 1. Repetitive UV absorbance scans recorded during the hydrolysis of 3c and 4 in pH 4.60, 0.01 M acetate buffer (μ = 0.50 M (KCl)). Cycle time is 60 s. The first few scans are labeled for clarity. (A) Repetitive-wavelength scan for 3c. The initial concentration of 3c is 5.0 × 10^{-5} M. (B) Repetitive-wavelength scan for 4. The initial concentration of 4 is 5.7 × 10^{-5} M.

by eq 2, a rate equation for three consecutive first-order processes. Two of the rate constants obtained from this

$$A_t = A_1e^{-k_1t} + A_2e^{-k_2t} + A_3e^{-k_3t} + A_\infty \quad (2)$$

treatment are identical within experimental error to k_0 and k' obtained at 266.5 nm. The third rate constant, k'', has a value of (6.42 ± 0.02) × 10^{-4} s^{-1} at this pH.

Similar results are obtained at all pH values in the range 1.0–8.0. Values of the three rate constants obtained under these conditions are presented in Table I, and plots of the logarithms of the rate constants vs. pH are shown in Figure 2A. The process described by k_0 is observable over the entire pH range. Its rate is independent of pH. The process described by k' is acid catalyzed at low pH and becomes too rapid at pH <3.0 to be measured by conventional methods. The process governed by k'' shows both acid- and base-catalyzed components, as well as a significant uncatalyzed rate. This process becomes too rapid at pH >6.0 to be observed. Similar kinetic results were obtained previously for the hydrolysis reactions of N-(pivaloyloxy)-p-methoxyacetanilide over a more limited pH range.[9]

Table II. Pseudo-First-Order Rate Constants for the Decomposition of 4 at 25 °C[a]

buffer[b]	pH[c]	k'',[d] s^{-1}
HCl	1.00	(6.39 ± 0.04) × 10^{-3}
HCl	1.30	(3.40 ± 0.02) × 10^{-3}
HCl	1.59	(1.90 ± 0.01) × 10^{-3}
HCl	2.00	(9.77 ± 0.02) × 10^{-4}
HCl	2.31	(7.03 ± 0.02) × 10^{-4}
HCl	2.62	(5.75 ± 0.02) × 10^{-4}

[a] Data on k' and k'' for 4 in the pH range 3.0–8.0 can be found in ref 9. [b] Ionic strength 0.50 M (KCl). Acetate and phosphate buffers were 0.01 M in total buffer. [c] ±0.02 at 25 °C. [d] At these pH values k' is too large to measure by conventional methods.

The repetitive wavelength scans of UV absorbance taken during the hydrolysis of N-acetyl-p-benzoquinone imine (4) under conditions identical to those for 3c are shown in Figure 1B. The characteristics of the time dependence of the UV spectra obtained for 4 are remarkably similar to those observed for 3c. The only significant difference between the two is the absence of the rapid initial process described by k_0 in the UV spectral scans for 4. We have previously shown that absorbance vs. time data obtained for 4 at 266.5 nm can be fit well by the standard first-order rate equation.[9] The rate constant obtained in this fashion is experimentally indistinguishable from k' measured for 3c. For example, at pH 5.64 in acetate buffer, k' for 3c is (3.94 ± 0.01) × 10^{-4} s^{-1}, while k' measured for 4 is (3.98 ± 0.03) × 10^{-4} s^{-1}.[9,10] Absorbance vs. time data taken at 245 nm for 4 can be fit to eq 1 to obtain two rate constants.[9] One of these is equivalent to k', while the other is indistinguishable from k'' measured for 3c. Table II presents kinetic data obtained for 4 in the pH range 1.0–2.6. Results obtained at higher pH have been previously published.[9] Figure 2B illustrates that k' and k'' measured for 4 are identical in magnitude and pH de-

(9) Novak, M.; Pelecanou, M.; Pollack, L. J. Am. Chem. Soc. 1986, 108, 112.
(10) A correction of −0.05 units should be applied to pH measurements given in ref 9 for comparison to this work. See the Experimental Section.
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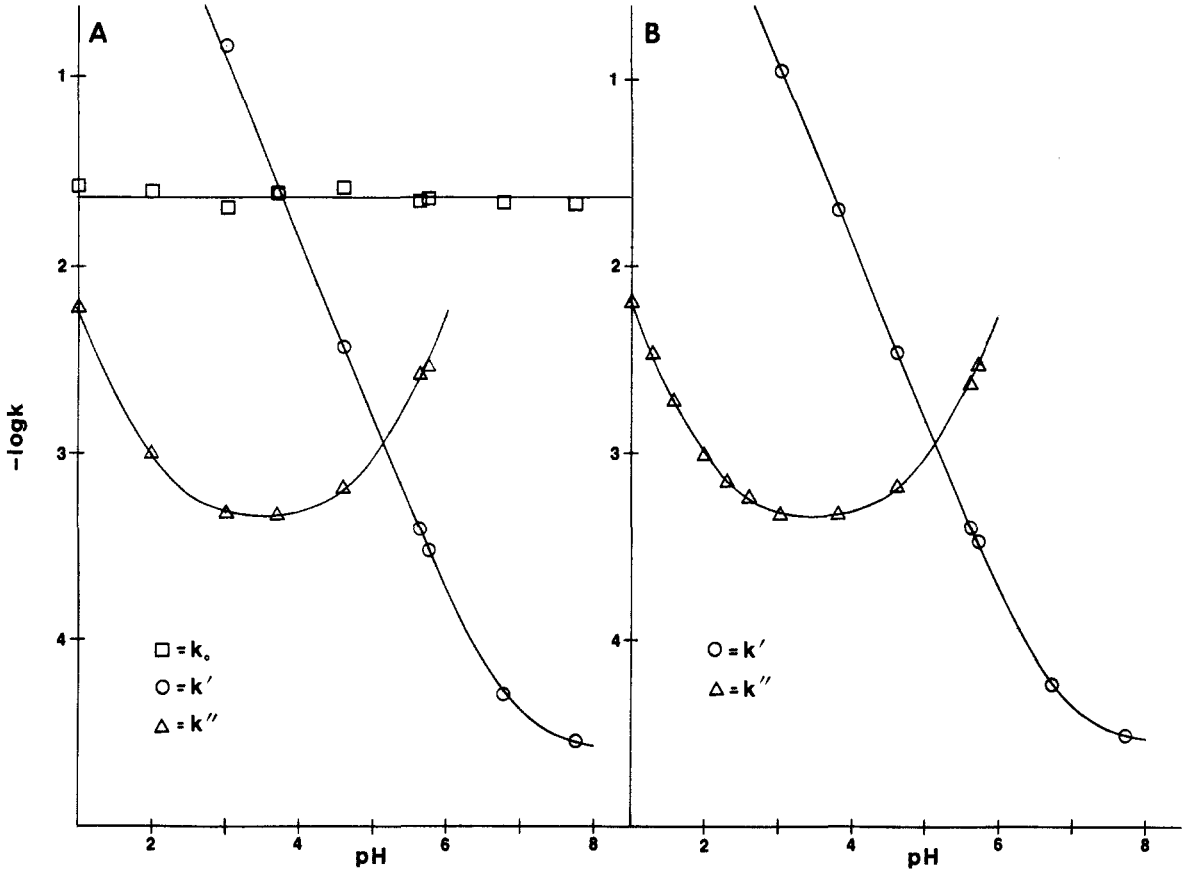


Figure 2. Plots of the logarithms of k_0, k', and k'' vs. pH for **3c** and **4**. Theoretical lines were obtained from least-squares fits as described in the text. (A) Plot for **3c**. Data were from Table I. (B) Plot for **4**. Data for this plot were obtained from Table II and ref 9.

Table III. Rate Parameters for the Decomposition of **3c** and **4** at 25 °C[a]

compd	10^2k_0, s^{-1}	$10^5k'_c$,[b] s^{-1}	k'_H,[b] M^{-1} s^{-1}	$10^4k''_c$,[c] s^{-1}	$10^2k''_H$,[c] M^{-1} s^{-1}	$10^{-5}k''_{OH}$,[c] M^{-1} s^{-1}
3c	2.36 ± 0.23	2.51 ± 0.10	107 ± 15	4.30 ± 0.06	5.32 ± 0.47	4.99 ± 0.46
4[d]		2.73 ± 0.07	96 ± 8	4.26 ± 0.04	5.72 ± 0.22	5.50 ± 0.30

[a] Rate parameters reported with their standard deviations. [b] Parameters derived from a weighted nonlinear least-squares fit of observed k' values to a modified form of eq 3: $k' = k'_c + k'_H[H^+]^x$. The exponent x was treated as a least-squares parameter. Best fit values of x were 0.97 ± 0.01 for **3c** and 0.96 ± 0.01 for **4**. [c] Parameters derived from a weighted nonlinear least-squares fit of observed k'' values to eq 4. [d] These values differ slightly from those reported in ref 9. In the earlier study pH readings were not corrected (see the Experimental Section), and since data were not obtained below pH 3.0, the k''_H term was not observed.

pendence to the corresponding rate constants measured for **3c**.

The pH dependence of k' and k'' can be expressed by eq 3 and 4, respectively. Table III lists the rate parameters

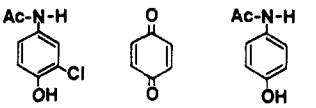
$$k' = k'_c + k'_H[H^+] \quad (3)$$

$$k'' = k''_c + k''_H[H^+] + k''_{OH}[OH^-] \quad (4)$$

derived for **3c** and **4** from a least-squares fit of k' data to eq 3 and k'' values to eq 4. The average value of k_0 for **3c** is also listed in Table III. The derived rate parameters for **3c** and **4** are equivalent within experimental error. Figure 2 shows that the fit of the data to eq 3 and 4 is excellent for both compounds.

It is evident that **4** is produced during the hydrolysis of **3c**. The magnitude of the absorbance changes observed for **3c** indicate that >90% of **3c** is converted into **4** during the course of the reaction at all pH values. The rate constant k_0 is associated with the decomposition of **3c**, while k' and k'' both describe processes associated with the decomposition of **4**.

Results of product studies performed by HPLC analysis of hydrolysis reaction mixtures of **4** at various pH have been reported.[9] At low pH the only products observed by this method are 3-chloro-4-hydroxyacetanilide (**5**) and *p*-benzoquinone (**6**). ^1H NMR analysis performed in D_2O confirmed that acetamide and **6** are formed in a 1/1 ratio in these reactions.[9] At higher pH acetaminophen (**7**) becomes a major product, and the combined yield of **5**, **6**, and **7** is signficantly less than quantitative. This is due to the



5 **6** **7**

formation of an oligomeric product that is apparently produced by the reaction of **4** and **7**.[9] Dahlin and Nelson have shown that **7** markedly increases the rate of decomposition of **4**.[11] They ascribed this to a comproportionation reaction between **4** and **7** which produces a semi-imino quinone radical that rapidly decomposes. We have also noted the effect of **7** on the rate of decomposition of **4**. In order to obtain pseudo-first-order rate constants for the decomposition of **4** at pH >6.0, it is necessary to work at very low concentrations (ca. 2.5×10^{-6} M) so that the subsequent reaction between **4** and its decomposition product **7** does not interfere.[9] Under these conditions the combined yields of the monomeric products **5**, **6**, and **7** approach the quantitative limit.[9] All the rate data reported in Table I at pH >6.0 were obtained under conditions in

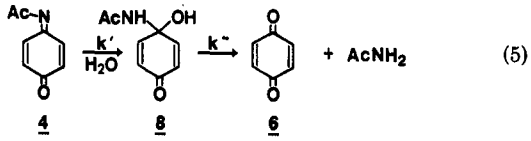
Table IV. Yields of Products Obtained from the Hydrolysis of 3c at 25 °C under Various pH Conditions

buffer[a]	pH	% yields[b] 5	6	7	1
HCl	3.0	27 ± 1	65 ± 5		
KOAc/HOAc	4.6	22 ± 1	65 ± 3		
K_2HPO_4/KH_2PO_4	5.7	14 ± 1	44 ± 3	8 ± 2	1.3 ± 0.1
K_2HPO_4/KH_2PO_4[c]	6.7	2.5 ± 0.5	17 ± 3	29 ± 3	2.2 ± 0.4
K_2HPO_4/KH_2PO_4[c]	7.7		6 ± 1[d]	35 ± 1	3.1 ± 0.5
K_2HPO_4/KH_2PO_4 [μ = 0.50 M (KI)]	6.7				98 ± 5

[a] Ionic strength 0.50 M (KCl, unless otherwise indicated). Acetate and phosphate buffers were 0.01 M in total buffer. Initial concentration of 3c was ca. 1.0×10^{-4} M. [b] Yields were determined by HPLC methods and are reported with respect to 3c initially present. Yields were determined after 10 half-lives of the slowest process reported in Table I. [c] Less than quantitative yields are due to the formation of another, oligomeric, product. See text and ref 9. [d] This is a lower limit, since significant decomposition of 6 occurs at this pH.

which the initial concentration of 3c was no greater than 2.5×10^{-6} M so that pseudo-first-order conditions would exist.

We have previously shown that the biphasic nature of the decomposition of 4 at pH <6.0 is due to the buildup and decay of the carbinolamide 8 (eq 5).[9] This material



4 8 6 (5)

can be detected by both ^1H NMR and HPLC techniques, which also show that 8 decomposes into 6 and acetamide.[9] The ring-chlorinated product 5 results from acid-catalyzed nucleophilic attack of Cl^- (KCl is used to maintain ionic strength at 0.50 M) on 4.[9] At the low buffer concentration used (0.01 M) no products due to nucleophilic attack by acetate or phosphate were detected. The mechanism for the formation of 7 in aqueous solutions of 4 is currently not understood.[9]

Product studies on the hydrolysis reactions of 3c are complicated by our inability to obtain analytically pure samples of this material. Therefore, the results reported in Table IV are subject to some error. Nevertheless, it is clear that the great majority of products are those expected from the decomposition of 4. The relative yields of 5, 6, and 7 derived from 3c are comparable to those obtained from 4.[9] The only product not attributable to 4 is phenacetin, 1, which is produced in moderate yields at pH >5.5. We have observed that other N-(pivaloyloxy)acetanilides give moderate yields of the corresponding acetanilides at neutral pH.[5c,9] The N-O-glucuronide, 3b, also gives about a 20% yield of 1 when incubated at 37 °C in pH 7.4 buffer.[4] In the presence of 0.5 M KI, 3c is quantitatively reduced to 1. No acetaminophen, 7, which is the only product of the decomposition of 4 under these conditions,[5d] is observed in these experiments. Evidently, KI traps a species involved in the decomposition of 3c at a point prior to the formation of 4. The glucuronide 3b yields ca. 27% of the rearrangement product 2-hydroxyphenacetin glucuronide when incubated at pH 7.4.[4] Small amounts of ring-substituted (pivaloyloxy)phenacetins are already present in the samples of 3c.[6] However, no increase in the concentration of these materials is observed when 3c undergoes hydrolysis. The quantitative trapping of the hydrolysis reaction by KI also indicates that rearrangement products are not formed during this reaction. We have shown previously that KI has no effect on the yield of such rearrangement products formed during the hydrolysis of ester derivatives of other N-hydroxyacetanilides.[5] If significant quantities of rearrangement products were formed we would not have observed quantitative formation of 1 in the presence of KI. At the low concentrations of acetate and phosphate used in this study, no products due to attack by these species were isolated.

At pH >3.5, 4 is stable enough to be detected by HPLC methods during the hydrolysis reactions of 3c. In fact, 3c decomposes so rapidly ($t_{1/2}$ = 29.4 s) that HPLC chromatograms of the hydrolysis mixtures taken after the first few minutes of reaction are almost exact duplicates of those previously reported for 4.[9] The only significant differences is that the hydrolysis mixtures of 3c at pH >5.5 contain small but detectable quantities of 1. This material is produced as rapidly as 3c decomposes. All other final products are produced at a rate consistent with the rate of decomposition of 4 or the carbinolamide 8, which can also be detected by HPLC methods in the pH range 3.5–5.5. The rates of formation and decomposition of 4 and 8, and the rate of decomposition of 3c, observed in the HPLC experiments are consistent with the kinetic data reported in Table I.

All of our data clearly show that the model phenacetin metabolite 3c rapidly decomposes predominately into the reactive intermediate N-acetyl-p-benzoquinone imine, 4. Over the entire pH range of the study at least 90% of the reaction pathway involves 4. Our results from this and previous studies[5,9] also provide considerable evidence concerning the mechanism of this process.

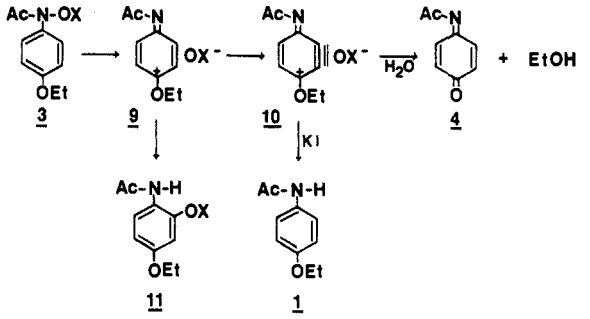
The hydrolysis rate constants of a series of ring-substituted N-(sulfonatooxy)acetanilides at 40 °C correlate with Brown's σ^+ parameter[12] to give a slope, ρ, of −4.5 ± 0.9.[5a] A similar correlation for a series of N-(pivaloyloxy)acetanilides at 70 °C gave a ρ value of −5.6 ± 0.8.[9] These large negative slopes, and the identity of the hydrolysis products, clearly indicate that ester derivatives of the N-hydroxyacetanilides decompose via a nitrenium ion[13] pathway in aqueous media.[5,9] The pivalic acid ester of N-hydroxyphenacetin, 3c, is no exception to this general observation. Like the other more reactive members of the two series, it decomposes in aqueous solution with a rate constant (k_0) that is pH independent. The value of k_0 for 3c ($(2.36 \pm 0.23) \times 10^{-2}$ s^{-1} at 25 °C) is (1.8 ± 0.2)-fold larger than the corresponding rate constant measured for the closely related compound N-(pivaloyloxy)-p-methoxyacetanilide under identical conditions.[9] The similarity in hydrolysis rates of these two compounds is certainly consistent with the nitrenium ion mechanism. The reduction of 3c to 1 in the presence of KI is also a characteristic reaction of compounds which hydrolyze via a nitrenium mechanism.[5]

The KI reduction has previously allowed us to distinguish between different types of ion pair intermediates involved in the hydrolysis of esters of the N-hydroxyacetanilides.[5] Tight nitrenium ion–counter ion pairs cannot be trapped by KI and lead exclusively to rearrangement products, while solvent-separated ion pairs are attacked by external nucleophiles and are trapped by KI to yield the corresponding acetanilides.[5] In the case of ester derivatives of N-hydroxyphenacetin we have found that

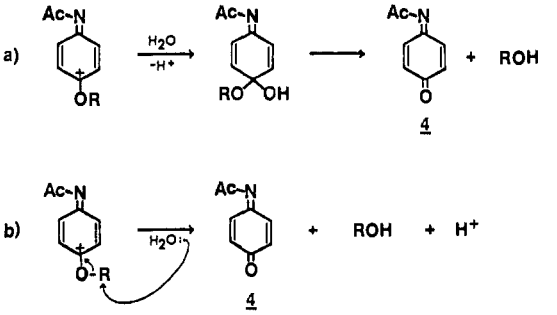
(12) Brown, H. C.; Okamoto, Y. J. Am. Chem. Soc. 1957, 79, 1913; 1958, 80, 4979.

(13) Gassman, P. G. Accts. Chem. Res. 1971, 3, 26. Gassman has also shown that the methanesulfonic acid esters of N-hydroxyacetanilides decompose by nitrenium ion pathways: Gassmann, P. G.; Granrud, J. E. J. Am. Chem. Soc. 1984, 106, 1498; 1984, 106, 2448.

Scheme I



Scheme II



3c yields no rearrangement products and can be quantitatively converted to 1 by KI. However, 3b yields significant amounts of the rearrangement product 2-hydroxyphenacetin glucuronide.[4] This difference can be understood in terms of the mechanism of Scheme I. We have previously shown that the lifetime of the tight ion pair, and hence the yield of rearrangement products, is influenced by two factors: the intrinsic stability of the nitrenium ion, which is governed by ring substituents, and the leaving group ability of XO^-.[5,9] As the nitrenium ion becomes more stable, and the leaving group gets better, the lifetime of the tight ion pair decreases and the yield of rearrangement product, 11, decreases. In the case of 3b and 3c, the nitrenium ion is quite stable so that if a good leaving group is available (3c) the lifetime of the tight ion pair, 9, is vanishingly short and detectable quantities of 11 are not formed. All the observed products arise from the solvent-separated ion pair, 10. The glucuronide anion is a much poorer leaving group than the pivalate ion ($t_{1/2}$ for 3b is 8.7 h at 37 °C compared to 29.4 s for 3c at 25 °C), so the tight ion pair has a longer lifetime in the case of 3b and the rearrangement product is formed in significant yield. We expect that the sulfate ester 3a would behave in a manner similar to 3c since sulfate is a better leaving group than the pivalate ion. In the absence of reducing agents 3a should be rapidly and quantitatively converted into 4. The small to moderate yields of 1 produced during the hydrolysis reactions of 3b and 3c in the absence of reducing agents may be due to competing homolytic processes that yield radicals or radical cations.[7,14] This seems quite likely, since the yield of 1 derived from 3b has been shown to be insensitive to nucleophiles, which apparently trap the nitrenium ion.[15] The greater yield of 1 derived from the compound with the poorer leaving group (3b) is also consistent with this supposition.[14]

Hinson and co-workers have provided suggestive evidence that 3b decomposes in aqueous solution to yield two deethylated electrophilic species.[15] One of these was presumed to be 4; the other was not identified. We have found no evidence for two such species in the reactions of 3c. The homolytic process referred to above may be responsible for this difference, since it appears to be important for 3b, but a very minor path for 3c.

It is clear from the KI trapping results that 4 is produced from the attack of H_2O on the solvent-separated ion pair 10. Two reasonable mechanisms for such a process are illustrated in Scheme II. Since both 3c and N-(pivaloyloxy)-p-methoxyacetanilide undergo hydrolysis at almost the same rate, and yield approximately the same proportion of 4,[9] mechanism a is more likely. Mechanism b would be expected to be considerably less efficient for the p-ethoxy compound 3c, but in fact, there is very little difference in the reactivity of the two compounds. Hinson, Nelson, and Gillette have previously obtained evidence based on the study of the hydrolysis of 3b in ^{18}O-enriched H_2O, which is also consistent with mechanism a.[16]

In conclusion, although indirect evidence for the involvement of N-acetyl-p-benzoquinone imine, 4, in the hydrolysis reactions of ester derivatives of 2, such as 3a or 3b, has been presented,[4,15] until now this elusive intermediate had not been directly detected during such reactions. We are able to detect 4 during the hydrolysis of 3c, and we can demonstrate that 4 accounts for at least 90% of the hydrolysis reaction of 3c in the pH range 1.0–8.0. The formation of N-hydroxyphenacetin, and its conversion into the reactive esters 3a and 3b, is apparently a minor pathway for the metabolism of phenacetin.[1-3,15,16] However, since these paths demonstrably lead to the highly toxic N-acetyl-p-benzoquinone imine, 4, they may be of considerable importance. Based on this work and our previous studies on the sulfate and pivalate esters of the N-hydroxyacetanilides,[5,9] we can predict that the sulfate ester 3a will decompose largely into 4, but its half-life under physiological conditions must be less than 1 s. Since 4 will not survive long in the presence of cellular nucleophiles and reducing agents,[9,11] it is almost certain that the effects of 3a are limited to the liver. The glucuronide 3b is stable enough to act at sites remote from the liver,[3,4] and it has been suggested that it may play a role in phenacetin-induced renal damage.[2] We can explain many of the differences in the reaction products of 3b and 3c in terms of lifetimes of tight and solvent-separated ion pairs. Other differences may be due to a homolytic pathway, which is more important for 3b due to the poorer leaving group ability of glucuronide anion.[7,14]

Experimental Section

Synthesis. N-Hydroxyphenacetin (2). This material was prepared by the reaction of N-hydroxy-p-phenetidine[17] with acetyl chloride in dry Et_2O according to published procedures.[5a] Physical and spectral properties of 2 were identical to those previously reported.[18]

N-(Pivaloyloxy)phenacetin (3c). This ester was prepared from 2 and pivaloyl chloride by a procedure previously used to make N-(pivaloyloxy)-4-methoxyacetanilide.[9] The yellow oily liquid, which was obtained as the crude product, was 90–95% pure by ^1H NMR and HPLC analysis. HPLC conditions were as follows: μ-Bondapak C-18 column, 7:3 MeOH/H_2O eluent, 1 mL/min, UV absorbance monitored at 250 nm. Attempts to purify this material led to significant decomposition. Spectral data were obtained from the unpurified material: IR (neat 2980, 2940, 1780, 1690, 1510, 1480, 1250 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 1.28 (9 H, s), 1.43 (3 H, t, J = 7.0 Hz), 2.03 (3 H, s), 4.05 (2 H,

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q, J = 7.0 Hz), 6.95 (2 H, d, J = 9.0 Hz), 7.38 (2 H, d, J = 9.0 Hz); MS, m/e (relative intensity) 279 (M^+, 7.2), 237 (10.7), 179 (5.0), 178 (5.6), 177 (11.5), 153 (39.0), 136 (16.8), 135 (73.5), 109 (10.0), 108 (13.8), 107 (26.3), 51 (100). Spectral properties are consistent with those previously reported for related compounds.[5c,7,9]

***N*-Acetyl-*p*-benzoquinone Imine (4).** This material was synthesized by the procedure of Dahlin and Nelson.[11]

Kinetic Measurements. All kinetics were performed in 5 vol % CH_3CN–H_2O at 0.50 M ionic strength (KCl). Procedures for purification of solvents, preparation of solutions, and general methods for following the progress of reactions by UV absorption spectroscopy have been described.[5a,b]

Kinetic measurements were performed at (25.0 ± 0.1) °C over the pH range 1.0–8.0 in HCl solutions and in acetate or phosphate buffers. All pH readings were taken at (25.0 ± 0.1) °C with an Orion Model 801 digital pH meter equipped with a Radiometer Model GK 2402C combination electrode. Measurements of pH on standardized HCl solutions in the pH range 1.0–3.0 established the following relationship between pH and the meter reading:

$$\text{pH} = \text{meter reading} - 0.05 \quad (6)$$

Equation 6 was used to correct all pH measurements.

Procedures used to monitor the hydrolysis kinetics of 4 have been published.[9] Identical procedures were used for **3c**. Methods for handling the kinetic data have also been described.[5a–c,9]

Product Analyses. Product studies were performed in solutions identical to those used in the kinetic studies except for higher concentrations of **3c** (ca. 1.0×10^{-4} M). The purity of **3c** was determined by NMR peak integration (ca. 90–95% pure). A correction based on this calculated purity was applied to all product yield data. Product yields were determined by HPLC methods that have been published.[9] All HPLC peak areas were calibrated with a known concentration of authentic samples, which were obtained commercially or prepared in previous studies.[5a]

Detection of 4 during the Hydrolysis of 3c by HPLC Methods. The hydrolysis of **3c** was initiated by the injection of 25 μL of a ca. 2.0×10^{-2} M solution of **3c** in CH_3CN into 5 mL of an appropriate buffer solution that had been incubating for sufficient time at (25.0 ± 0.1) °C. Aliquots of 10 μL were periodically removed and injected directly onto an HPLC column (μ-Bondapak C-18 column, 1:1 MeOH/H_2O eluent, 1 mL/min, UV absorbance monitored at 250 nm). HPLC peaks for **4** (rt = 4.3 min) and its labile hydrolysis product **8** (rt = 3.1 min) could be observed at appropriate times and pH values. These materials decomposed in a manner identical to that previously reported for authentic **4** and **8**.[9] No other intermediates were observed during the hydrolysis of **3c**.

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Synthesis of [[(Naphthalenylmethoxy)- and [[(Quinolinylmethoxy)phenyl]amino]oxoalkanoic Acid Esters. A Novel Series of Leukotriene D_4 Antagonists and 5-Lipoxygenase Inhibitors

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A series of novel [[(naphthalenylmethoxy)- and [[(quinolinylmethoxy)phenyl]amino]oxoalkanoic acid esters have been prepared. These compounds were tested as inhibitors of rat polymorphonuclear leukocyte (PMN) 5-lipoxygenase (LO) in vitro and as inhibitors of ovalbumin (OA) and leukotriene D_4 (LTD_4) induced bronchospasm in the guinea pig (GP) in vivo. Many naphthalenyl compounds were potent inhibitors of 5-LO, and several quinolinyl compounds were potent inhibitors of LTD_4-mediated bronochospasms in the GP. The most potent naphthalenyl compound, 4-[[3-(2-naphthalenylmethoxy)phenyl]hydroxyamino]-4-oxobutanoic acid, methyl ester (**6v**), had an IC_{50} of 0.6 μM in the 5-LO assay. The most potent compound in vivo, 4-[[3-(2-quinolinylmethoxy)phenyl]hydroxyamino]-4-oxobutanoic acid, methyl ester (**6e**), had ED_{50}'s of 3.3 mg/kg and 27.4 mg/kg (intraduodenally) against LTD_4- and OA-induced bronchospasm, respectively. When tested as an antagonist of LTD_4-induced contraction of isolated GP tracheal spiral strips, **6e** was shown to be a competitive inhibitor with a pK_B value of 5.33.

Leukotrienes (LT), products of the 5-lipoxygenase (LO) biosynthetic pathway, may play an important role in the pathophysiology of such disease states as asthma, psoriasis, ulcerative colitis, and rheumatoid arthritis.[1,2] The sulfidopeptide LTs, LTC_4, LTD_4, and LTE_4, previously referred to as slow-reacting substance of anaphylaxis (SRS-A), are known to contract lung smooth muscle and evoke bronchoconstriction in the guinea pig (GP) and man.[3] In humans, LTC_4 and LTD_4 also stimulate the release of mucus from airways in vitro,[4] are potent vasodilators in skin,[5] and produce a wheal and flare response when injected intradermally.[6] The nonpeptidic LT, LTB_4, is an extremely potent chemotactic, and aggregating agent for a variety of leukocytes in vitro; in vivo, it stimulates cell accumulation and increases vascular permeability.[7] The multiple actions and potency of LTs have prompted the development of agents that inhibit the formation or action of these substances.

FPL-55,712 (**1**) (Figure 1) was the first compound to be described as an antagonist of sulfidopeptide LTs.[8] As a pharmacological tool, it has been extensively used to define

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