Cyclohexadienes from the rearrangement of *O*-aroyl-*N*-acetyl-*N*-(2,6-dimethylphenyl)hydroxylamines. Reaction in aqueous solution to *meta*- and *para*-substituted 2,6-dimethylacetanilides

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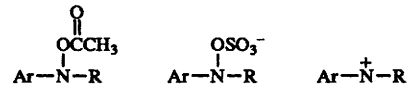
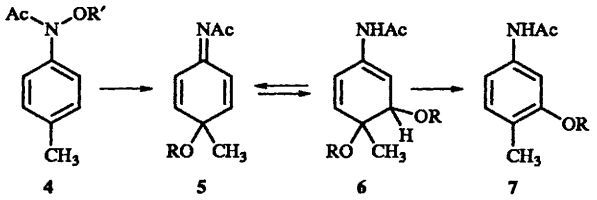
O-Aroyl-*N*-acetyl-*N*-(2,6-dimethylphenyl)hydroxylamines (aroyl = benzoyl, 3-nitrobenzoyl, and pentafluorobenzoyl) rearrange in acetonitrile to 1,5-dimethyl-5-aroyloxy-6-*N*-acetyliminocyclohexa-1,3-dienes that can be isolated as pure compounds. These cyclohexadienes react in aqueous solutions, producing *m*-aroyloxy- and *m*-hydroxy-2,6-dimethylacetanilides in an H^+-catalysed reaction and the corresponding *para* products in a non-catalysed reaction. Analysis of the effect of the aroyloxy group suggests that the latter reaction involves heterolysis to a reactive, non-selective, ion pair that collapses to the *para* product, or reacts at this position with water. The *meta* products arise from the *N*-protonated cyclohexadiene reacting with solvent in a conjugate addition to give the *meta*-substituted phenol, or in an intramolecular reaction with the carbonyl group to give the rearranged ester. This latter reaction is proposed to proceed *via* an intermediate 1,3-dioxolan-2-ylium ion. The nucleophiles azide, phenylsulfinate and the methyl thioglycolate anion react in a bimolecular fashion to give *meta*-substituted 2,6-dimethylacetanilides. With phenylsulfinate this requires H^+, while methyl thioglycolate anion reacts with the neutral cyclohexadiene. Azide exhibits reaction by both processes. These reactions are proposed to involve conjugate additions, either to the *N*-protonated cyclohexadiene, or, with better nucleophiles, directly on the neutral compound. These cyclohexadienes model intermediates that may form during the metabolism of certain carcinogenic amines. These results establish the presence of three electrophilic species capable of reacting with cellular nucleophiles—the highly reactive and non-selective cation formed in the heterolysis, the less reactive and more selective *N*-protonated species, and the neutral cyclohexadiene itself. The last electrophile is relatively unreactive, but can be a target of very good nucleophiles such as thiol anions.

The carcinogenicity of aromatic amines is believed to be associated with their metabolic oxidation to the hydroxylamine level, followed by conversion into *O*-acetates **1** or *N*-sulfates **2**. Arylnitrenium ions **3**, obtained upon N–O ionization of these esters, are postulated as the ultimate carcinogens that interact with cellular components.[1–3]



1 R = H, $COCH_3$ **2** **3**

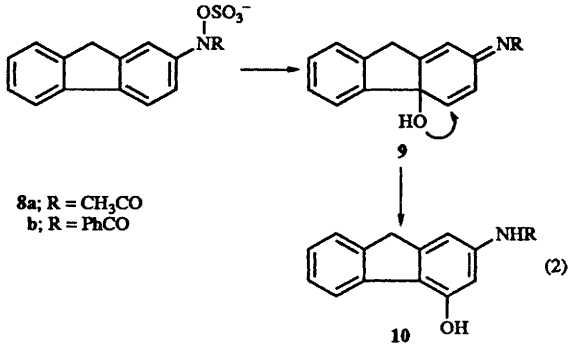
A number of investigations of the chemistry of esters of these types have been carried out.[2–12] In general, ionization produces ion pairs or free ions that react with nucleophiles at the *ortho* and *para* position of the aromatic ring. When this ring position bears a hydrogen atom, aromatization occurs *via* loss of this proton to give the appropriate *ortho* or *para*-substituted aniline derivative. In cases where the ring position is substituted (*e.g.*, **4**), *meta*-substituted products (*e.g.*, **7**) can be obtained. These



4 **5** **6** **7**

a; R = CH_3, R′ = SO_2CH_3
b; R = H, R′ = SO_3^- (1)

have been proposed to arise through conjugate addition to the original adduct followed by elimination, a sequence demonstrated by Gassman and Granrud through the isolation of **5a** and two isomers of **6a** in the reaction of **4a** in the presence of methanol.[6] Novak and Roy have provided additional evidence through the NMR characterization of **5b** and **6b** in the hydrolysis of **4b**.[9] In the widely studied fluoren-2-yl system, the hydroxy adduct **10** is formed,[4] and the intermediate **9a** has been observed with NMR during the hydrolysis of the *N*-sulfate **8a**.[10] Recent oxygen-18 tracer experiments with an independently synthesized *N*-benzoyl derivative **9b** have demonstrated that **10** forms in an intramolecular reaction.[12]



8a; R = CH_3CO
b; R = PhCO

9 **10** (2)

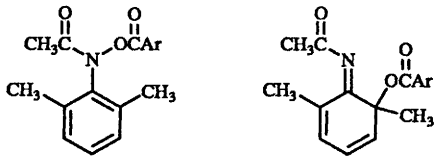
These examples have involved an external nucleophile adding to a substituted *para* position to form an intermediate 3-iminocyclohexa-1,4-diene. In a study of the 2,6-dimethyl derivatives **11a–c**, we observed that stock solutions in acetonitrile rearrange to give cyclohexa-1,3-dienes **12a–c**, which could be isolated as pure solids. These products arise *via* addition at a substituted *ortho* position by the leaving group originally attached at nitrogen, and the effect of this group on the subsequent chemistry can be readily probed. The cyclohexa-1,3-dienes are in fact sufficiently long-lived in aqueous solution

Table 1 Rate constants for the decomposition of 1,5-dimethyl-5-(aroyloxy)-6-acetyliminocyclohexa-1,3-dienes **12a–c** in aqueous solutions. Conditions: 40 °C and ionic strength 1 mol dm^{-3} in 8.3% by volume acetonitrile.

Aroyloxy	pK_a(ArCOOH)[a]	k_H/dm^3 mol^{-1} s^{-1}	k_o/s^{-1}
C_6H_5COO–	4.20	2.92×10^3	2.5×10^{-5}
3-$NO_2C_6H_4COO$–	3.45	5.38×10^2	2.0×10^{-4}
C_6F_5COO–	1.75	1.55×10^2	1.56×10^{-2}

[a] pK_a of the conjugate acid of the aroyloxy leaving group.

for a detailed examination of their chemistry, and that is the subject of this paper. In the following paper, we discuss the behaviour of **11** in water. This is a more complex system reacting in part by way of the isomers **12**.



11 **12**

a Ar = C_6H_5
b Ar = 3-$NO_2C_6H_4$
c Ar = C_6F_5

Results

Kinetics in the absence of added nucleophiles

The cyclohexadienes **12a–c** possess maxima in absorbance near 325 nm, and their reactions in buffered aqueous solution were easily monitored by following the decrease at this wavelength. Studies were carried out in formate, acetate, dihydrogen phosphate and triethanolamine buffers containing 8.3% by volume acetonitrile, with the temperature 40 °C and the ionic strength at 1 mol dm^{-3} being maintained by $NaClO_4$. Excellent first-order behaviour was observed in all cases. Rate accelerations by the buffers were not observed, the observed rate constants being unchanged (±3%) in experiments at constant pH and buffer concentrations below 0.05 mol dm^{-3}. At higher buffer concentrations the rate constants were actually observed to decrease, by as much as 30% in some cases. The nature of this effect was not investigated in detail; it may be associated with a specific salt effect. In most cases the buffer-independent first-order rate constant was simply taken as the rate constant measured at low buffer concentration. In other cases extrapolation to zero buffer concentration was carried out.

The dependence of the rate constant on pH is shown in Fig. 1. The data are consistent with the two-term rate law given in eqn. (3) in which there is a change with decreasing pH from a pH-

$$k_{obs} = k_o + k_H[H^+] \quad (3)$$

independent to an H^+-dependent reaction. Values of the rate constants k_o and k_H that give the best fit to the experimental data are given in Table 1.

Products in the absence of added nucleophiles

Product analyses were carried out under conditions identical with those employed for the kinetic experiments. Products were identified as the esters **13** or **14** retaining the aryloxy group, the phenols **15** or **16** and the appropriate benzoic acid. Conditions where the kinetics show dominance of the H^+-dependent process result only in *meta* products **13** and **15**, while the *para*-

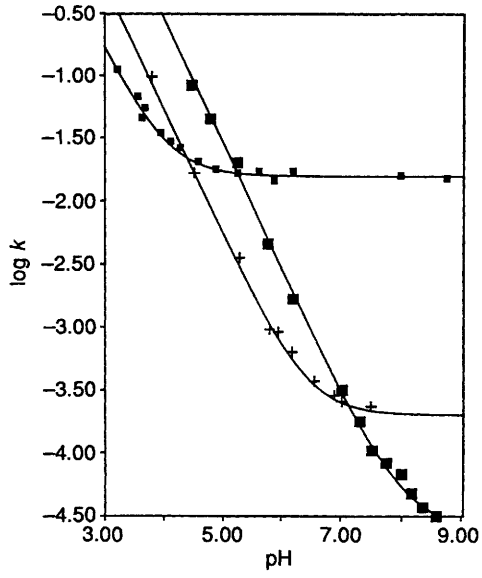
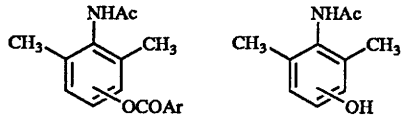


Fig. 1 pH-Dependence of observed rate constants for the disappearance of 1,5-dimethyl-5-(aroyloxy)-6-acetyliminocyclohexa-1,3-dienes: (■) **12a**, benzoyloxy; (+) **12b**, *m*-nitrobenzoyloxy; (■) **12c**, pentafluorobenzoyloxy (k_{obs} in units of s^{-1}, at 40 °C and ionic strength 1 mol dm^{-3} in 8.3% by volume acetonitrile)



13 *meta* **15** *meta*
14 *para* **16** *para*

substituted **14** and **16** are obtained when the pH-independent reaction dominates. Control experiments demonstrated that, in all but one case, the phenol plus benzoic acid combination did not arise from the further hydrolysis of the ester. That one exception was the pH-independent hydrolysis of the *m*-nitrobenzoyloxy compound, in which a slow hydrolysis did occur under conditions required for complete disappearance of the starting material. This, however, could be circumvented by carrying out product analyses after only partial conversion.

Quantitative results are summarized in Table 2. Within experimental uncertainties, the products with the pentafluoro and *m*-nitro derivatives quantitatively accounted for the starting material, yields summing to 103% and 93%, respectively, for the pH-independent reactions, and 102% for both of the acid reactions. Moreover, the yields of phenol and benzoic acid were, to within experimental error, identical in each of these systems. With the benzoyloxy derivative, the identified products for the H^+-dependent reaction accounted for 87% of the starting material, and moreover the yields of phenol product and benzoic acid were not identical. We were unable to trace the source of this discrepancy or to locate other products. This compound could not be studied under the conditions where the pH independent reaction was the sole process, because of the dominance of the acid process even at high pH. Analyses carried out under different experimental conditions revealed that, for a particular reaction, product distributions were not measurably affected by changes in pH or buffer concentration.

Kinetics—reactions with nucleophiles

The disappearance of **12** was studied under the standard reaction conditions in the presence of three nucleophiles—

Table 2 Products obtained from the reactions of 1,5-dimethyl-5-(aroyloxy)-6-acetyliminocyclohexa-1,3-dienes **12** in aqueous solutions[a]

Product	$C_6H_5COO^-$ (**12a**)[b]	3-$NO_2C_6H_4COO^-$ (**12b**)[c]	$C_6F_5COO^-$ (**12c**)[d]
H^+-dependent reaction			
meta ester **13**	0.796 ± 0.012	0.833 ± 0.025	0.436 ± 0.006
meta phenol **15**	0.048 ± 0.004	0.187 ± 0.009	0.578 ± 0.009
ArCOOH	0.104 ± 0.007	0.193 ± 0.017	0.581 ± 0.023
pH-independent reaction			
para ester **14**		0.593 ± 0.022	0.462 ± 0.015
para phenol **16**		0.34[e]	0.566 ± 0.012
ArCOOH		0.34[e]	0.565 ± 0.008

[a] For reactions at 40 °C, 1 mol dm^{-3} ionic strength ($NaClO_4$) in 8.3% by volume acetonitrile. Yields were determined by HPLC analysis after 10 half-lives of reaction, unless noted otherwise, and are reported as fractions based upon the number of moles of reacted starting material. Errors represent one standard deviation, based upon replicate measurements. [b] Products of the H^+-dependent reaction were determined from six experiments, five in 0.005 mol dm^{-3} total acetic acid, 50% in the acetate form, and one in 0.025 mol dm^{-3} acetic acid, 50% acetate. Products of the pH-independent reaction were not analysed owing to interference from the H^+-dependent process. [c] Products of the H^+-dependent reaction were determined from nine experiments, eight in 0.0183 mol dm^{-3} acetic acid, 10% acetate, and one on 0.092 mol dm^{-3} acetic acid, 10% acetate. Products of the pH-independent reaction were determined from two experiments in 0.066 mol dm^{-3}, 97% HPO_4^{2-}, at 25% conversion of the starting material. [d] Products of the H^+-dependent reaction were based on seven experiments, six in 0.0041 mol dm^{-3} $HClO_4$ and one 0.010 mol dm^{-3} $HClO_4$. Products of the pH-independent reaction were determined from five experiments, three in 0.016 mol dm^{-3} phosphate buffer, 30% HPO_4^{2-}, one in 0.066 mol dm^{-3} phosphate, 97% HPO_4^{2-}, and one in 0.097 mol dm^{-3} acetic acid, 95% acetate. [e] Only the ester product could be accurately determined in these experiments. Yields of **16** and ArCOOH were calculated with the assumption that these were equal and that the phenol and ester products total 93% (see the Experimental section).

sodium azide, sodium phenylsulfinate and methyl thioglycolate. In experiments carried out at constant pH, the observed rate constants were found to increase in a linear fashion with increasing concentration of nucleophile,

$$k_{obs} = k_{int} + k_{Nuc}(obs) \times [Nuc] \quad (4)$$

where k_{int} is the rate constant at that particular pH in the absence of the nucleophile. There was no change in k_{obs} in experiments in which the nucleophile concentration and the pH were held constant and the concentration of the buffer employed to maintain pH (usually phosphate) was varied.

Experiments with sodium azide were carried out in phosphate buffers where azide exists predominantly in its conjugate base form. The second-order rate constants k_{Nuc}, however, did depend on pH, a plot *versus* $[H^+]$ being linear with a significant intercept [eqn. (5)]. Thus, there are two pathways, a direct reaction of azide anion with the substrate, plus one that is catalysed by H^+.

$$k_{Az}(obs) = k_o(Az) + k_H(Az) \times [H^+] \quad (5)$$

With phenylsulfinate, which was also studied at pH values where the anion predominants, k_{Nuc} was again linear in H^+, but the intercept was, to within experimental error, zero.

$$k_{PS}(obs) = k_H(PS) \times [H^+] \quad (6)$$

For methyl thioglycolate, k_{Nuc} increased with increasing pH up to the point of ionization of the thiol [pK_a(RSH) = 8.04], following rate law (7). Thus, in this case there was a direct

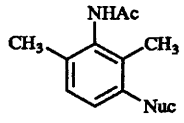
$$k_{RS}(obs) = k_o(RS) \times \left(\frac{K_a(RSH)}{K_a(RSH) + [H^+]}\right) \quad (7)$$

reaction of the anion with the substrate, while the H^+-catalysed process observed with the other two nucleophiles was, to within experimental error, negligible.

Values of the rate constants k_o(Nuc) and k_H(Nuc) are given in Table 3.

Products—reactions with nucleophiles

The products resulting from the reaction with phenylsulfinate, methyl thioglycolate and the H^+-dependent reaction with azide were isolated from scaled-up reactions, and were identified as 3-substituted-2,6-dimethylacetanilides **17**–**19**. Analysis by HPLC



17 –Nuc = – N_3
18 –Nuc = – $SO_2C_6H_5$
19 –Nuc = – SCH_2COOCH_3

showed that, with one exception, this 3-substituted anilide was the only additional species observed in the presence of the nucleophile. Moreover, the relative amount of this product was, to within experimental error, that expected on the basis of the rate increase observed with the added nucleophile.

The exception to this behaviour occurred with the 3-nitrobenzoyloxy and pentafluorobenzoyloxy compounds in their reactions with azide at high pH. These are conditions where azide reacts directly with the substrate, and, in the absence of the nucleophile, the substrate undergoes an uncatalysed decomposition. Here the *meta* adduct **17** was present, but there was an additional product observed in the HPLC traces. Unfortunately reaction conditions could not be found where this was present in sufficient quantity to be isolated and characterized.

Discussion

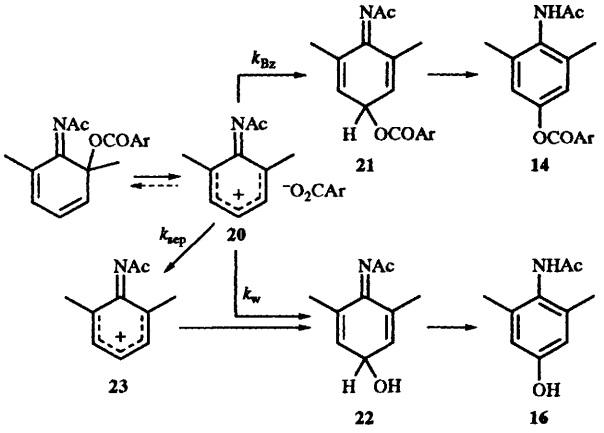
The cyclohexa-1,3-dienes **12** can clearly react to give products where nucleophiles have substituted on a ring carbon in positions *meta* and *para* to the original position of attachment of the aroyloxy group in the hydroxylamine derivative. *meta*-Substitution is the result of an H^+-catalysed reaction, and also occurs in the reactions with the added nucleophiles. *para*-Substitution is the result of a pH-independent process.

pH-independent reaction

Our proposed mechanism for the formation of the *para* products is shown in Scheme 1. This involves a non-catalysed heterolysis of the C–O bond resulting in an ion pair **20** that

Table 3 Rate constants for the reactions of 1,5-dimethyl-5-(aroyloxy)-6-acetyliminocyclohexa-1,3-dienes **12a–c** with added nucleophiles. Conditions: 40 °C and ionic strength 1 mol dm^{-3} in 8.3% by volume acetonitrile

Nucleophile	Aroyloxy	k_H(Nuc)/ $dm^6\ mol^{-2}\ s^{-1}$	k_o(Nuc)/ $dm^3 mol^{-1} s^{-1}$
N_3^-	$C_6H_5CO_2-$	7.1×10^5	$< 5 \times 10^{-2}$
	$3\text{-}NO_2C_6H_5CO_2-$	6.1×10^5	2.5×10^{-2}
	$C_6F_5CO_2-$	3.4×10^5	5.0×10^{-2}
$PhSO_2^-$	$C_6H_5CO_2-$	7.6×10^4	
	$C_6F_5CO_2-$	3.8×10^4	
$MeOOCCH_2S^-$	$C_6H_5CO_2-$		2.4×10^2



Scheme 1

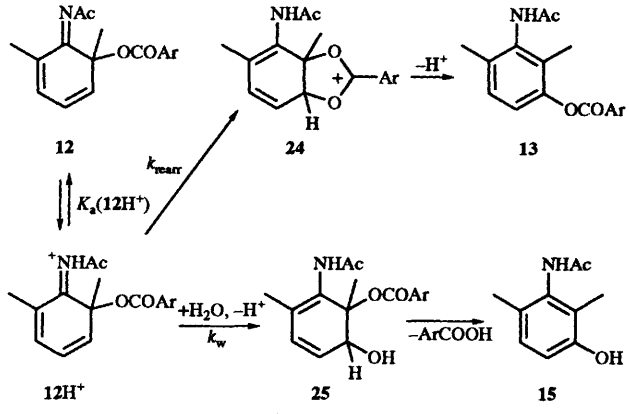
collapses to give the isomer **21** that tautomerizes to the ester **14**. Competing with the internal rearrangement is reaction with solvent resulting in **22**, the precursor to the phenol **16**. The reaction with water could also occur at the stage of the free ion **23**. Although the intermediate cation is written as a conjugated carbenium ion, another resonance contributor is an *N*-arylnitrenium ion, the same nitrenium ion obtained by N–O heterolysis of the hydroxylamine derivative **11**. The nature of the ions and ion pairs formed in these heterolysis processes is discussed in detail in the accompanying paper.

The ion-pair intermediate is established by the observation that in the absence of added benzoate, products are still formed involving transfer of the leaving group. A direct concerted rearrangement could also explain this, but such a mechanism is inconsistent with the large dependence of k_o on Ar. A plot of log k_o *versus* pK_a(ArCOOH) has a slope, β_{lg}, of -1.1, implying a substantial accumulation of negative charge on the leaving group in the rate-limiting transition state. This is consistent with the ionization step being rate-limiting, with a late transition state for C–O bond cleavage, or with rate-limiting capture of the cation. In the latter case, the observed β_{lg} is a composite of the value for the equilibrium formation of the cation and the effect on the capture. The former is expected to be large and negative, while the latter is likely to be quite small since the transition state for the reaction with nucleophiles is expected to be early and non-selective (see below).

For the two compounds studied under neutral conditions, the ester : phenol ratio **[14]** : **[16]** shows only a small dependence on the leaving group, changing from 1.74 for 3-nitrobenzoate to 0.82 for pentafluoro, while the basicity of the benzoate varies by a factor of 50. For the mechanism where solvent capture occurs after separation of the ions, these ratios correspond to $k_{Bz}:k_{sep}$. The latter represents a diffusional separation and should be relatively insensitive to the aroyloxy anion. In this case therefore, the small effect can be interpreted as arising from a slight dependence for k_{Bz}, the less basic pentafluorobenzoate trapping the cation in the ion pair approximately two times slower than 3-nitrobenzoate. For the mechanism where solvent capture occurs at the ion-pair stage, this product ratio is determined by $k_{Bz}:k_w$. In this case the small dependence can be explained by a reactive, non-selective, cation in the ion pair. This is consistent with the low azide : water selectivity previously observed with the *N*-(2,6-dimethylphenyl)nitrenium ion, the analogue of **23** lacking the *N*-acetyl group.[13]

H^+-dependent reaction

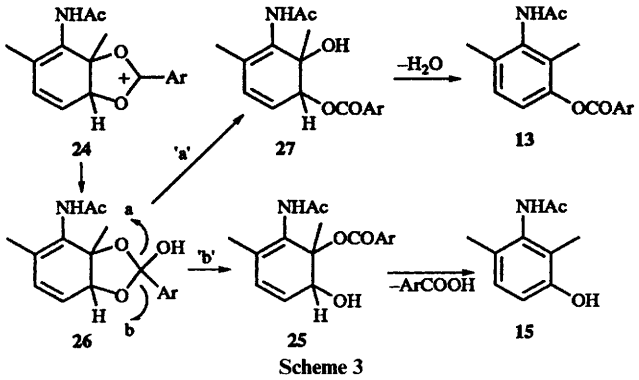
Mechanisms that account for the products that form under acid conditions are given in Scheme 2. This reaction is specific-acid



Scheme 2

catalysed since buffers have no effect on the rate, and thus a likely intermediate is the protonated acylimine **12H**$^+$ forming reversibly from the neutral substrate. Reaction of this cation with water results in the adduct **25**, which is set up for the elimination of ArCOOH, thus accounting for the phenol **15**. This pathway is in common with the acid-catalysed additions of azide and phenylsulfinate, as will be discussed later. The ester **13** is proposed to arise through a similar sequence involving the carbonyl oxygen of the aroyloxy group acting as an internal nucleophile resulting in an intermediate 1,3-dioxolan-2-ylium ion.[14,15] Although this class of cation normally adds nucleophiles at the 2-position,[16,17] our proposed mechanism with **24** involves deprotonation concomitant with ring opening to produce directly the aromatic product. The restoration of the aromatic resonance could well lower the energy barrier for deprotonation relative to water addition, much in the same way that formation of an aromatic system changes the rate-limiting step in alcohol dehydration.[18]

Addition of water to the dioxolan-2-ylium ion, if it were to occur, would give the intermediate **26**, and, as shown in Scheme 3, this would be expected to go on to both products. That this does not occur is indicated by the very different dependencies of



Scheme 3

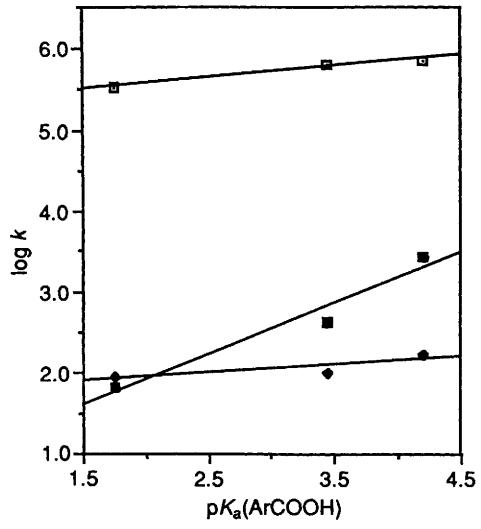
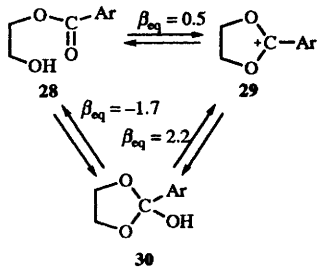


Fig. 2 Dependence of log k_H(phenol) (◆), log k_H(ester) (■) and log k_H(Az) (⊡) on the pK_a of the aroyloxy leaving group. Slopes of the lines are 0.09, 0.63 and 0.13, respectively. Values of k_H(phenol) and k_H(ester) have been calculated by multiplying the observed k_H (Table 1) by the fractions of *meta*-substituted phenol and *meta*-substituted ester (Table 2), respectively.

the products upon the aryl substituent. That the aryl substituent could markedly affect the partitioning of the ring-opening reaction seems unlikely, particularly in such a way as is required to explain the dependence of the product ratio.

The dependence of the products on the aryl group is illustrated in Fig. 2, in the form of plots of log k for the two processes as a function of pK_a(ArCOOH). The reaction forming the *meta*-phenol sees only a small effect, with the Brønsted-like plot having a slope β of 0.09. According to our proposed mechanism, the rate constant for this reaction is the product of k_w and $1/K_a(12H^+)$. Such a β is consistent with a small effect of Ar on the acidity constant. The position of protonation is three atoms removed from the –OCOAr group, and thus the β for the protonation step should be $1 \times (\sim 0.45)^3$ where the number in parentheses represents the fall-off factor associated with each intervening atom.[19] The intramolecular process on the other hand has a significant dependence, with a β value of 0.63. This is a clear indication of build-up of positive charge on the carbon adjacent to the aryl ring, and is obviously consistent with a mechanism involving rate-limiting formation of a 1,3-dioxolan-2-ylium ion.

A system that models the cyclization of **12** to **24** is the conversion of the ester **28** into the cation **29**. This reaction can be demonstrated to exhibit a substituent effect, in this case on the overall equilibrium constant, that is of a similar order of magnitude (Scheme 4). The β_{eq} value in this case has been obtained by combining the β of +2.2 calculated from data for the equilibrium conversion of **30** into **29**[20] with a β of −1.7

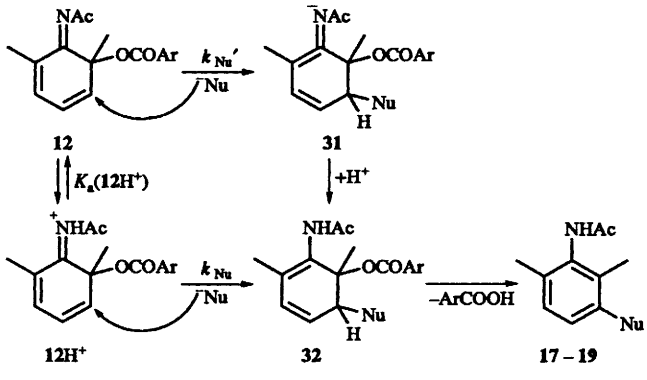


Scheme 4

for **28** ⟶ **30**, the latter typical of the formation of tetrahedral adducts of aroyl derivatives.[21,22] The relatively low β values for these cyclizations forming 1,3-dioxolan-2-ylium ions arise through a combination of two effects. The positive charge in the cation is delocalized to the two oxygens, and this attenuates the interaction with aryl substituents. In addition, the starting ester has a significant interaction of the aryl substituents and the positive end of the C=O dipole,[21,22] so that the placement of a full positive charge has a smaller effect, relative, for example, to the reaction where the cation forms from a neutral compound such as **30**.

Reactions with added nucleophiles

Nucleophiles accelerate the decay of the cyclohexadienes, producing *meta*-substituted products that account, to within experimental error, for the rate acceleration. With azide, and, especially with the methyl thioglycolate anion, this reaction occurs on the neutral cyclohexadiene. Azide also reacts in an H^+-catalysed reaction, as does the phenylsulfinate anion. Based upon the common site of attachment of the three nucleophiles a general mechanism as summarized in Scheme 5



Scheme 5

can be proposed. In this mechanism nucleophiles react by conjugate addition to the C=C affording intermediates that can aromatize by loss of ArCOOH. With strong nucleophiles such as the thiol anion, and, to a lesser extent, azide, this reaction occurs on the neutral substrate, producing the conjugate base of the amide as the initial intermediate. With poorer nucleophiles such as phenylsulfinate, prior activation by *N*-protonation is required for the addition to occur. The absence of buffer catalysis implies that none of the proton transfer steps is rate-limiting or concerted with the formation of the bond between the nucleophiles and the carbon. The acid reaction is analogous to that proposed in Scheme 3 to account for the *meta*-substituted phenol. As shown in Fig. 2, there is a very similar substituent dependence for this latter reaction and the H^+-catalysed azide addition, consistent with this interpretation.

The experiments with added nucleophiles were originally carried out in an attempt to trap the ion pair (or free cation) of the non-catalysed reaction (Scheme 1). The expectation is that

Table 4 Rate constants for the reactions of protonated 1,5-dimethyl-5-(aroyloxy)-6-acetyliminocyclohexa-1,3-dienes with added nucleophiles, calculated with the assumption that azide reacts with $k_{az} = 5 \times 10^9$ dm^3 mol^{-1} s^{-1}

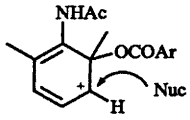
Substrate	k_w/s^{-1}	k_{rearr}/s^{-1}	$k_{PhSO_2^-}$/ dm^3 mol^{-1} s^{-1}
12aH$^+$ (C_6H_5)	1.1×10^6	1.9×10^7	5.3×10^8
12bH$^+$ (3-$NO_2C_6H_4$)	8.2×10^5	3.5×10^6	
12cH$^+$ (C_6F_5)	1.3×10^6	9.6×10^5	5.5×10^8

this would yield a product with the nucleophile substituted at the position *para* to the acetylamino group, but with no rate acceleration. The bimolecular reactions however proved to be too competitive, and conditions could not be attained where the cyclohexadiene reacted by heterolysis to the ion pair, and yet at the same time there was enough of the nucleophile present in the solution to divert this ion pair from reacting with the counterion and solvent. The experiments with azide at high pH did in fact show an additional product, but conditions could not be found where this could be isolated for identification.

Acidity constant and lifetime of protonated cyclohexadiene

The observed rate constants k_H(Az) and k_H(phenol) are equal to the quantities k_{Az}/K_a(**12**H$^+$) and k_w/K_a(**12**H$^+$) where k_{Az} and k_w refer to the reactions of the nucleophiles with the protonated cyclohexadiene and K_a(**12**H$^+$) is the acidity constant of the protonated cyclohexadiene. The ratios k_H(Az):k_H(phenol) are 4.3×10^3 (**12a**), 6.0×10^3 (**12b**) and 3.9×10^3 (**12c**), probably within experimental error the same, particularly considering that k_H(phenol) is obtained as the product of an observed rate constant and the fraction of phenol product. Since the K_a term is common to the two expressions for k_H, these ratios represent k_{Az}:k_w, and thus measure the competition between azide and water for the protonated cyclohexadiene.

This cation can be viewed as a resonance stabilized carbocation [see (**12**H$^+$)′]. There has been considerable



(**12**H$^+$)′

discussion of azide:water selectivities for carbocations,[23–25] and the above values for **12**H$^+$ fall in the range where azide is reacting at the diffusion limit. In particular these selectivities are three–four orders of magnitude lower than those observed for highly stabilized cations that react with azide in an activation-controlled process.[26,27] With the assumption of diffusion control for azide, and taking k_{Az} as 5×10^9 dm^3 mol^{-1} s^{-1},[24,25] the observed k_H(Az) require pK_a values of −3.84 (**12a**H$^+$), −3.91 (**12b**H$^+$) and −4.16 (**12c**H$^+$). Acidity constants for protonated acylimines have not been measured, but it is expected that these compounds should be strongly acidic due to the presence of the electron-withdrawing acyl group, coupled with the loss on protonation of the resonance interaction between the nitrogen and carbonyl group. Novak and co-workers have estimated the effects of these two factors to predict the pK_a of protonated *N*-acetyl-*p*-benzoquinone imine as -5.7 ± 2.[28] The values for **12**H$^+$ fall within this range.

With values of K_a(**12**H$^+$) available, rate constants for the other acid-catalysed reactions can be calculated, and these are provided in Table 4. These cations are relatively short-lived with lifetimes of the order of 50 ns to 1 μs dependent on the nature of the aromatic substituent.

Selectivities of electrophiles in cyclohexadiene systems

Although nitrenium ions have been postulated as the electrophile responsible for DNA-binding of aromatic amines, the short lifetime of these cations raises concerns over their biological role.[13] This has led to some speculation that the hydroxylamine ester itself could be the electrophile, undergoing a reaction with DNA in an S_N2 manner.[29–32] Interestingly, very recent investigations have shown that the nitrenium ions derived from 2-aminofluorene and *p*-aminobiphenyl do have a significant lifetime in water[11,33] and, moreover, can react selectively in water with guanine.[34]

Many carcinogenic amines have substituents at the *ortho* or *para* position, and thus, in principle, their hydroxylamine derivatives can form relatively long-lived cyclohexadiene intermediates analogous to the ones studied here. While a biological role for such species has not been demonstrated, they can react in three different electrophilic forms. Moreover these electrophiles are of quite different reactivity and selectivity towards nucleophiles. The most reactive and least selective is the carbenium ⟷ nitrenium cation. With some possible exceptions,[11,33,34] such cations are unlikely to survive long enough to react with DNA unless formed in the immediate proximity. These cations may also exist with different degrees of ion pairing, with some ion pairs incapable of reaction with external nucleophiles (next paper). A second electrophile is the *N*-protonated cyclohexadiene, which, as demonstrated by this study, is a viable intermediate at physiological pH despite its low concentration. This intermediate could be particularly important at pH 7 for cyclohexadienes bearing a benzoate (as in **12a**) or acetate where the heterolysis reaction would be slow. As discussed in the last paragraph, this electrophile is also highly reactive, although it would appear to be less reactive and more selective than the cation produced in the heterolysis. Moreover, since it forms by equilibrium protonation of the neutral cyclohexadiene, the possibility exists that the latter could diffuse through the cell, with the protonated species forming and reacting in the vicinity of DNA. Finally, the neutral cyclohexadiene is itself electrophilic. This species is long-lived in water alone, and does not undergo direct nucleophilic attack by the solvent and weak nucleophiles. It does, however, react with powerful nucleophiles, and a biological role could be envisaged involving thiol anions in cells.

Experimental

Materials

All chemicals were reagent grade or better and, with the exception of the ones described below, were commercially available. Inorganic materials were used without further purification and organic chemicals were typically recrystallized or distilled before used. Water and methanol employed in the kinetic experiments and for HPLC analysis were distilled in glass.

N-Acetyl-*N*-(2,6-dimethylphenyl)hydroxylamine

This was prepared by the reaction of *N*-(2,6-dimethylphenyl)-hydroxylamine[35] with acetyl chloride according to a literature procedure.[36] This material had mp 126–128 °C; δ_H ($CDCl_3$; 200 MHz) 1.83 (3 H, s), 2.26 (6 H, s) and 7.05–7.30 (3 H, m) (Found: M$^+$, 179.0951. Calc. for $C_{10}H_{13}NO_2$; *M*, 179.0947).

O-Aroyl-*N*-acetyl-*N*-(2,6-dimethylphenyl)hydroxylamines were prepared by the reaction of the anion of the *N*-acetylhydroxylamine with the appropriate benzoyl chloride in aqueous tetrahydrofuran (THF) at 0 °C. In a typical procedure, 8.2 cm^3 of 3.92 mol dm^{-3} aqueous NaOH (32 mmol) were added at 0 °C under a stream of nitrogen to a rapidly stirred solution containing 6 g (33.5 mmol) of *N*-acetyl-*N*-(2,6-dimethylphenyl)-

hydroxylamine dissolved in 250 cm^3 of THF. To the resulting emulsion was added dropwise over 5 min pentafluorobenzoyl chloride (4.8 cm^3, 33.3 mmol) dissolved in 50 cm^3 of THF. To this reaction mixture 200 cm^3 of ether were added and the aqueous phase was discarded. The organic phase was extracted twice with 50 cm^3 of saturated aqueous $NaHCO_3$, dried two times each with Na_2SO_4 and $MgSO_4$, after which the solvent was removed on a rotary evaporator. Pure product was isolated from unchanged starting material by flash chromatography using 50:50 hexane–ethyl acetate as the eluent, followed by concentration and recrystallization from ethyl acetate–hexane. The yield of purified material was 3.7 g (37%).

The NMR spectra of these esters were consistent with the existence of two populations of amide rotamers, and in the listings below the chemical shifts due to the minor isomer, where determinable, are listed in parentheses following the chemical shift values of the major isomer.

***O*-Benzoyl-*N*-acetyl-*N*-(2,6-dimethylphenyl)hydroxylamine (11a).** Mp 123–125 °C, $\delta[(CD_3)_2SO$; 200 MHz] 1.86 (3 H, s), (2.25), 2.43 (6 H, s) (2.25), 7.10–7.38 (3 H, m), 7.52–7.64 (2 H, d), 7.72 (1 H, t) and 7.95–8.08 (2 H, m) (Found: C, 71.7; H, 6.35; N, 5.0. Calc. for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94%).

***O*-(3-Nitrobenzoyl)-*N*-acetyl-*N*-(2,6-dimethylphenyl)hydroxylamine (11b).** Mp 121–122 °C, $\delta[(CD_3)_2SO$; 200 MHz] 1.95 (3 H, s) (2.31), 2.50 (6 H, s) (2.46), 7.17 (2 H, d) (7.10), 7.28 (2 H, t), 7.66 (1 H, t) (7.72), 8.36 (1 H, d), 8.42 (1 H, d) (8.48) and 8.84 (1 H, s).

***O*-(Pentafluorobenzoyl)-*N*-acetyl-*N*-(2,6-dimethylphenyl)-hydroxylamine (11c).** Mp 79–79.5 °C, $\delta[(CD_3)_2SO$; 200 MHz] 1.88 (3 H, s) (2.29), 2.35 (6 H, s) (2.30) and 7.16–7.41 (3 H, m) (Found: C, 54.6; H, 3.4; N, 3.6; F, 25.2. Calc. for $C_{17}H_{12}F_5NO_3$: C, 54.70; H, 3.24; N, 3.75; F, 25.45%).

1,5-Dimethyl-5-aroyloxy-6-*N*-acetyliminocyclohexa-1,3-dienes **12** were prepared by refluxing **11** in dry acetonitrile until more than 99% of the starting material had disappeared, as monitored by HPLC. This approach of consuming completely the hydroxylamine ester was necessary since chromatography failed to separate the cyclohexadiene from the starting material. Although **12** is the only product formed from **11** in 100% acetonitrile, significant decomposition occurs in the time required for complete reaction. The further products can however be easily separated. Suitable reflux times depended on the aryl substituent, being 70 min, 9 h and 6 days for the pentafluorobenzoyloxy, 3-nitrobenzoyloxy, and unsubstituted benzoyloxy derivatives, respectively. Yields of isolated **12** were 30–40%.

In a typical procedure, 1 g of *O*-benzoyl-*N*-acetyl-*N*-(2,6-dimethylphenyl)hydroxylamine was refluxed for 134 h in 150 cm^3 of acetonitrile that had been freshly distilled from calcium hydride. The solvent was removed on a rotary evaporator, and the remaining material was subjected to flash chromatography on silica gel with 1:1 ethyl acetate–hexane as the eluent. Fractions giving a single spot on TLC (silica gel; 1:1 ethyl acetate–hexane, R_F = 0.66) were pooled and the solvent was removed to give *ca.* 0.5 g of an oil that was recrystallized from 1:10 ethyl acetate–hexane to yield 0.3 g (30%) of the pure cyclohexadiene.

1,5-Dimethyl-5-(benzoyloxy)-6-acetyliminocyclohexa-1,3-diene (12a). Mp 94.5–95.5 °C, $\delta_H[(CD_3)_2SO$; 200 MHz] 1.74 (3 H, s), 1.86 (3 H, s), 1.97 (3 H, s), 5.93 (1 H, d), 6.12 (1 H, dd), 6.57 (1 H, d), 7.46 (2 H, t), 7.57 (1 H, t) and 8.02 (2 H d). ^{13}C $\delta_C[(CD_3)_2SO]$ 17.38, 25.28, 25.98, 78.73, 120.74, 128.95, 129.10, 129.36, 131.51, 133.52, 133.86, 137.49, 162.09, 164.24 and 180.97 (Found: C, 72.1; H, 6.1; N, 4.95. Calc. for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94%).

1,5-Dimethyl-5-(3-nitrobenzoyloxy)-6-acetyliminocyclohexa-1,3-diene (12b). Mp 133.5–134 °C, $\delta[(CD_3)_2SO$; 200 MHz] 1.74 (3 H, s), 1.94 (3 H, s), 1.97 (3 H, s), 5.93 (1 H, d), 6.15 (1 H, dd), 6.58 (1 H, d), 7.66 (1 H, t), 8.32 (1 H, d), 8.42 (1 H, d) and 8.81 (1 H, s) (Found: C, 61.92; H, 4.99; N, 8.92. Calc. for $C_{17}H_{16}N_2O_5$: C, 62.19; H, 4.91; N, 8.53%).

1,5-Dimethyl-5-(pentafluorobenzoyloxy)-6-acetyliminocyclohexa-1,3-diene (12c). Mp 87–87.5 °C, $\delta[(CD_3)_2SO$; 200 MHz] 1.63 (3 H, s), 1.97 (3 H, s), 2.14 (3 H, s), 5.96 (1 H, d), 6.16 (1 H, dd) and 6.54 (1 H, d) (Found: C, 54.45; H, 3.25; N, 4.0. Calc. for $C_{17}H_{12}F_5NO_3$: C, 54.70; H, 3.24; N, 3.75%).

3,5-Dimethyl-4-acetamidophenol was prepared by the addition at 0 °C of 0.6 cm^3 of acetyl chloride to 2.3 g (17 mmol) of 3,5-dimethyl-4-aminophenol dissolved in 150 cm^3 of dry ether. After precipitation of the unchanged hydrochloride of the starting amine, 0.3 g of sodium carbonate and 10 cm^3 of water were added. After 5 min, an additional 0.9 cm^3 of acetyl chloride was added and the reaction was allowed to stir a further 20 min, during which time a significant amount of precipitate appeared. To the reaction mixture were added 100 cm^3 of Bu^{t}OH and 50 cm^3 of water, the resulting two phases were separated and the aqueous phase was discarded. The solvents were stripped off using a rotary evaporator, and the remaining solid was recrystallized twice from ethyl acetate. 3,5-*Dimethyl*-4-*acetamidophenol* (**16**): mp 184–184.5 °C, $\delta_H[(CD_3)_2SO]$ 1.97 (3 H, s), 2.01 (6 H, s), 6.42 (2 H, s), 8.91 (1 H, s) and 9.10 (1 H, s); $\delta_C[(CD_3)_2SO]$ 18.22, 22.50, 114.06, 126.77, 136.17, 155.29 and 168.05 (Found: M^+, 179.0931. Calc. for $C_{10}H_{13}NO_2$: *M*, 179.0946).

3,5-Dimethyl-4-acetamidophenyl benzoates were prepared by reacting the oxyanion of 3,5-dimethyl-4-acetamidophenol with the appropriate benzoyl chloride in THF, using a method analogous to that described above for the synthesis of the *O*-aroyl-*N*-acetyl-*N*-(2,6-dimethylphenyl)hydroxylamines. 3,5-*Dimethyl*-4-*acetamidophenyl benzoate* (**14a**): mp 189–190 °C, $\delta[(CD_3)_2SO]$ 2.04 (3 H, s), 2.15 (6 H, s), 6.99 (2 H, s), 7.60 (2 H, t), 7.75 (1 H, t), 8.11 (2 H, d) and 9.24 (1 H, s) (Found: C, 71.7; H, 6.5; N, 5.0. Calc. for $C_{17}H_{16}NO_3$: C, 72.07; H, 6.05; N, 4.94%). 3,5-*Dimethyl*-4-*acetamidophenyl* 3-*nitrobenzoate* (**14b**): mp 121–121.5 °C, $\delta[(CD_3)_2SO]$ 2.05 (3 H, s), 2.16 (6 H, s), 7.03 (2 H, s), 7.91 (1 H, t), 8.5–8.6 (2 H, m), 8.76 (1 H, t) and 9.27 (1 H, s). 3,5-*Dimethyl*-4-*acetamidophenyl pentafluorobenzoate* (**14c**): mp 243–244 °C, $\delta[(CD_3)_2SO]$ 2.05 (3 H, s), 2.16 (6 H, s), 7.02 (2 H, s) and 9.30 (1 H, s) (Found: C, 54.55; H, 3.5; N, 3.7; F, 25.2. Calc. for $C_{17}H_{12}F_5NO_3$: C, 54.70; H, 3.24; N, 3.75; F, 25.45%).

3,5-Dimethyl-4-acetamidophenol and its pentafluorobenzoate were also isolated and characterized from a scale-up solvolysis in neutral aqueous solution of 1,5-dimethyl-5-(pentafluorobenzoyloxy)-6-*N*-acetyliminocyclohexa-1,3-diene (**12c**). The cyclohexadiene (0.2 g) in 10 cm^3 of acetonitrile, was added dropwise at 40 °C to a stirred solution of 150 cm^3 of water containing 10 cm^3 of acetonitrile and 10 cm^3 of 0.066 mol dm^{-3} phosphate buffer (40:60 HPO_4^{-2}–$H_2PO_4^-$). The reaction was stirred for 20 min, after which time sodium chloride was added to the saturation point and the reaction mixture was extracted three times with 100 cm^3 of ethyl acetate and two times with 100 cm^3 of Bu^{t}OH. The organic phases were pooled, dried two times with sodium sulfate and one time with magnesium sulfate, and most of the solvent removed with a rotary evaporator. Flash chromatography on silica gel with ethyl acetate as the eluent yielded two products, the more rapidly eluting one being 3,5-dimethyl-4-acetamidophenyl pentafluorobenzoate **14c**, followed by 3,5-dimethyl-4-acetamidophenol **16**. For both compounds, the appropriate fractions were pooled and concentrated and the remaining material recrystallized from ethyl acetate. These products were identical with the substances prepared as described above with respect to mp, NMR, exact MS, as well as showing identical retention times on silica gel TLC and HPLC.

2,4-Dimethyl-3-acetamidophenol and its pentafluorobenzoate ester were isolated from a scaled-up solvolysis in acidic solution of 1,5-dimethyl-5-(pentafluorobenzoyloxy)-6-acetyliminocyclohexa-1,3-diene **12c**. The diene, 3.2 g in 20 cm^3 of dry acetonitrile, was added dropwise at room temperature to a stirred solution containing 400 cm^3 of water, 50 cm^3 of acetonitrile and 3 cm^3 of 70% $HClO_4$. Initial cloudiness was followed by the development of a white precipitate. After 20 min, 100 cm^3 of ethyl acetate were added, followed by 6 g of Na_2HPO_4, and the mixture was extracted two times with 300 cm^3 of ethyl acetate and two times with 200 cm^3 of Bu^{t}OH. The organic phases were pooled, dried as described in the previous paragraph, and the solvent removed. The remaining material was a yellow viscous liquid containing some crystals, for which TLC on silica gel with 70:30 ethyl acetate–hexane showed two major products. The crystals in this mixture could be isolated by careful washing with ethyl acetate–hexane, followed by recrystallization from ethyl acetate–hexane to yield 2,4-*dimethyl-3-acetamidophenyl pentafluorobenzoate* **13c**: mp 205.5–206.5 °C, $\delta_H[(CD_3)_2SO]$ 2.00 (3 H, s), 2.06 (3 H, s), 2.16 (3 H, s), 7.08 (1 H, d), 7.18 (1 H, d) and 9.41 (1 H, s); $\delta_C[(CD_3)_2SO]$ 11.58, 17.95, 22.54, 119.78, 127.46, 134.05, 136.74, 146.46, 157.18 and 168.01. (Insufficient material was collected to observe the ^{13}C signals for the carbons in the pentafluorobenzene ring.) (Found: M^+, 373.0748. Calc. for $C_{17}H_{12}NO_3F_5$: *M*, 373.0738). The mother liquors from the recrystallization were combined with the original washings, concentrated, and subjected to flash chromatography on silica gel with ethyl acetate as eluent. Recrystallization from ethyl acetate–hexane gave 2,4-*dimethyl-3-acetamidophenol* **15**: mp 195.5–196.5 °C, $\delta_H[(CD_3)_2SO]$ 1.91 (3 H, s), 2.00 (6 H, s), 6.61 (1 H, d), 6.81 (1 H, d), 9.07 (1 H, s) and 9.13 (1 H, s); δ_C 11.21, 17.66, 22.60, 112.81, 121.84, 125.09, 126.78, 136.11, 153.59 and 167.81 (Found: M^+, 179.0940. Calc. for $C_{10}H_{13}NO_2$: M, 179.0947).

2,4-Dimethyl-3-acetamidophenyl 3-nitrobenzoate (13b)

This was prepared in the same manner, from the solvolysis under acidic conditions of 1,5-dimethyl-5-(3-nitrobenzoyloxy)-6-acetyliminocyclohexa-1,3-diene **12b**. This material had mp 197–198 °C, $\delta_H[(CD_3)_2SO]$ 1.97 (3 H, s), 2.05 (3 H, s), 2.16 (3 H, s), 7.12 (1 H, d), 7.17 (1 H, d), 7.92 (1 H, t), 8.59 (2 H, t), 8.79 (1 H, s) and 9.40 (1 H, s); $\delta_C[(CD_3)_2SO]$ 11.64, 17.99, 22.55, 120.07, 124.19, 127.58, 127.74, 128.42, 130.35, 131.00, 133.52, 135.81, 136.56, 147.03, 148.03, 162.74 and 167.98 (Found: M^+, 328.1063. Calc. for $C_{17}H_{16}N_2O_5$: *M*, 328.1060).

2,4-Dimethyl-3-acetamidophenyl benzoate (13a)

This was prepared by the reaction of 2,4-dimethyl-3-acetamidophenol with benzoyl chloride, as described above for **14a**. This material had mp 182–183 °C, $\delta[(CD_3)_2SO]$ 1.95 (3 H, s), 2.05 (3 H, s), 2.15 (3 H, s), 7.05 (1 H, d), 7.15 (1 H, d), 7.63 (2 H, t), 7.78 (1 H, d), 8.15 (2 H, d) and 9.37 (1 H, s).

2,6-Dimethyl-3-azidoacetanilide (17)

This was isolated from a scaled-up reaction of sodium azide and 1,5-dimethyl-5-(3-nitrobenzoyloxy)-6-acetyliminocyclohexa-1,3-diene **12b**. The diene, 0.6 g in 50 cm^3 of acetonitrile, was added dropwise at room temperature to a stirred solution containing 36 g of sodium azide and 1.4 cm^3 of 70% $HClO_4$ in 400 cm^3 of water. After 5 min, the reaction mixture was extracted with 300 cm^3 of ethyl acetate, and the organic phase was washed with 1:1 aqueous Na_2CO_3–$NaHCO_3$, followed by drying (Na_2SO_4 and $MgSO_4$) and removal of the solvent. The product was purified by recrystallization (ethyl acetate–hexane), followed by sublimation at reduced pressure. Mp 170–170.5 °C (decomp., with evolution of gas), $\delta[(CD_3)_2SO]$ 1.99 (3 H, s), 2.04 (3 H, s), 2.12 (3 H, s), 7.06 (1 H, d), 7.15 (1 H, d) and 9.14 (1 H, s) (Found: M^+, 204.1022. Calc. for $C_{10}H_{12}N_4O$: *M*, 204.1013).

2,6-Dimethyl-3-(phenylsulfonyl)acetanilide (18)

This was isolated by adding 1,5-dimethyl-5-(pentafluorobenzoyloxy)-6-acetyliminocyclohexa-1,3-diene **12c**, 0.12 g in 10 cm^3 of acetonitrile, to 50 cm^3 of water containing 3 cm^3 of 1.95 mol dm^{-3} acetic acid buffer (10% basic form), 16 g of sodium benzenesulfinate, and 10 cm^3 of acetonitrile. After 5 min of stirring at room temperature, the reaction mixture was extracted with 500 cm^3 of ethyl acetate, the organic phase was washed with aqueous $NaHCO_3$ and water, dried (Na_2SO_4 and $MgSO_4$) and the solvent removed leaving a white solid. This was purified by recrystallization from ethyl acetate–hexane. Mp 173–174 °C, $\delta[(CD_3)_2SO]$ 2.01 (3 H, s), 2.16 (3 H, s), 2.19 (3 H, s), 7.40 (1 H, d), 7.58–7.74 (3 H, m), 7.68 (2 H, d), 7.80 (1 H, d) and 9.37 (1 H, s) (Found: M^+, 303.0942. Calc. for $C_{16}H_{17}NSO_3$: *M*, 303.0930).

(2,4-Dimethyl-3-acetamidophenylthio)acetic acid methyl ester (19)

This was isolated by adding 1,5-dimethyl-5-benzoyloxy-6-acetyliminocyclohexa-1,3-diene **12a**, 0.4 g in 10 cm^3 of acetonitrile, to a stirred solution of 100 cm^3 of water, 20 cm^3 of acetonitrile, 0.38 g of methyl thioglycolate and 0.18 cm^3 of 1.92 mol dm^{-3} NaOH. Precipitation occurred after the first few drops of the diene had been added. Five minutes after the completion of the addition, 20 g of sodium chloride was added and the reaction mixture was extracted with 100 cm^3 of ethyl acetate. The organic phase was washed with cold aqueous K_2CO_3, dried ($MgSO_4$) and the solvent removed. The desired product was isolated from a considerable amount of unchanged starting material by chromatography on silica gel with ethyl acetate as the eluent, followed by three recrystallizations from ethyl acetate–hexane. Mp 113–114 °C, $\delta[(CD_3)_2SO]$ 2.03 (3 H, s), 2.09 (3 H, s), 2.15 (3 H, s), 3.60 (3 H, s), 3.80 (2 H, s), 7.05 (1 H, d), 7.15 (1 H, d) and 9.31 (1 H, s) (Found: M^+, 267.0939. Calc. for $C_{13}H_{17}NSO_3$: *M*, 267.0930).

Kinetics

Kinetic experiments were carried out by monitoring the decrease in the absorbance of the diene at the absorption maximum of 325 nm, using a Pye-Unicam SP 1800 spectrophotometer equipped with a water-jacketed compartment connected to a thermostatting bath. Reactions were carried out at 40.0 ± 0.2 °C with the ionic strength maintained at 1 mol dm^{-3} using $NaClO_4$. Reactions were initiated by adding 0.25 cm^3 of a diene solution in acetonitrile to 2.75 cm^3 of an aqueous solution such that the final concentration of the substrate was *ca.* 0.0001 mol dm^{-3}. In the case of the pentafluorobenzoyloxy derivative, it proved necessary to keep the stock solution of the substrate in acetonitrile in a dry ice–acetonitrile bath to prevent decomposition of the substrate. Stock solutions of the other substrates were kept on ice between use, and were stored on dry ice between different days. The stock solutions were checked by HPLC at regular intervals, these analyses showing less than 1% decomposition of the diene provided a given solution had been employed for less than four injections.

Rate constants were obtained from reactions carried out under pseudo-first-order conditions, with added nucleophile in excess over diene. Reactions were followed for 10 to 15 half-lives and rate constants were calculated by one of two methods: (*a*) from semi-logarithmic plots of $A_t - A_\infty$ *versus* time (linear for three–five half-lives) or (*b*) by a non-linear least-squares fitting

routine for a single exponential. When both methods were employed for the same data the agreement was better than ± 5%. In some reactions with higher diene concentrations (0.0002 mol dm^{-3}) there was a slow upward drift in the reaction end-point around seven half-lives associated with the appearance of slight turbidity in the solution, and a Guggenheim fitting routine was therefore employed. For the benzoate derivative **12a** at high pH where the reaction was very slow, first-order rate constants were determined by initial rate studies. For the first 10% of the reaction, plots of absorbance *versus* time were linear, and the slopes were divided by the substrate concentration to obtain the first-order rate constant.

Measurements of pH were made after the completion of the reactions using a Corning Model 130 pH meter equipped with a Fisher 'AccupHast' electrode containing 5 mol dm^{-3} lithium trifluoroacetate as internal electrolyte. The pH measurements were carried out at the same temperature as the reaction using a thermostatted titration vessel attached to a circulating water bath. The hydrogen-ion concentration was calculated from the equation $[H^+] = 10^{-[pH(obs)-0.10]}$, which was empirically determined by measuring the pH of solutions of known H^+ concentration containing 8.3% by volume acetonitrile and at 1 mol dm^{-3} ionic strength.

Reactions with methyl thioglycolate were carried out with solutions that were prepared in a glove bag under a nitrogen atmosphere. In these reactions a freshly prepared stock solution of the thiol was added to the cuvette containing the diene to initiate the kinetic measurements.

Product analysis

HPLC separations and quantitative determinations of products were carried out with a Varian LC 5500 chromatograph equipped with a diode array detector and a Model 601 data system. Typically an 0.200 cm^3 injector loop was overloaded with 0.25 cm^3 of a reaction solution and the products eluted on a Waters C-18 Spherisorb 15 cm column at a flow rate of 1.2 cm^3 min^{-1}. For the unsubstituted and pentafluoro derivatives, product separations were achieved using a linear gradient consisting of 5% methanol and 80% methanol, both solutions containing 0.26 mol dm^{-3} ammonium acetate. For the *m*-nitrobenzoyloxy derivative these conditions resulted in the co-elution of two of the products, the free acid (*m*-nitrobenzoic acid) and the *para* phenol obtained in the pH-independent reaction (3,5-dimethyl-4-acetamidophenol). These products however could be separated using the same gradient with the methanol–water solutions containing 1% acetic acid and no ammonium acetate.

Products were determined under the same conditions as employed for the kinetic runs, in most cases after a time corresponding to 10–15 half-lives of the reaction. Reacting solutions were directly introduced into the HPLC apparatus. Quantitative measurements were based upon standard curves constructed with the authentic samples prepared or isolated as described above.

In the case of the pH-independent reaction of the 3-nitrobenzoyloxy derivative, product analysis was carried out after 25% conversion of the starting material. This was necessary since experiments at 9 and 18 half-lives showed that a significant amount of hydrolysis of the ester product of this reaction (3,5-dimethyl-4-acetamidophenyl 3-nitrobenzoate) had occurred. The use of partially solvolysed solutions required that the eluting buffer contained 0.26 mol dm^{-3} ammonium acetate, since the starting material proved unstable in the 1% acetic acid buffer that provided the best separation in this system. As noted above *m*-nitrobenzoic acid and 3,5-dimethyl-4-acetylaminophenyl 3-nitrobenzoate co-eluted under these conditions. Their yields were therefore calculated with the assumptions (*a*) that they were equivalent and (*b*) that the total of 3,5-dimethyl-4-acetamidophenol and 3,5-dimethyl-4-acetamidophenyl 3-nitrobenzoate accounted for 93% of the starting material. The 93% figure was based upon observations after 9 and 18 half-lives (with acetic acid as the eluting buffer), which showed that although the ester had partially been converted into the phenol, the two products in total accounted for this quantity of material in each case.

Acknowledgements

R. A. M. acknowledges the continued support of the Natural Sciences and Engineering Research Council of Canada. Also acknowledged is the support of the North Atlantic Treaty Organization and the National Institutes of Health in the form of research fellowships to J. C. F.

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Paper 4/06946C
Received 14*th November* 1994
Accepted 28*th November* 1994