Sulphinamoylacetates as Sulphine Precursors. Mechanism of Basic Hydrolysis and Scheme of Irreversible Inactivation of Cinnamoyl Alcohol Dehydrogenase, an Enzyme of the Lignification Process

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t-Butyl *N*-arylsulphinamoylacetates are hydrolysed in aqueous basic media by an elimination mechanism. It takes place on the conjugate base of the substrate resulting from a fast deprotonation of the activated methylene group. This elimination is first order (*E*1cB) for compounds where strong electron-withdrawing groups substitute the aromatic ring. For substitution by weaker electron-withdrawing or -donating groups, the elimination is second order, and needs the presence of general acid catalysis. For t-butyl *N*-(2-hydroxyphenyl)sulphinamoylisobutyrate where this mechanism of elimination is impossible, a $B_{Ac}2$ attack by hydroxide ion at the sulphur atom takes place. From these mechanisms and the results of complexation of zinc(II) cations by sulphinamoylacetates, a possible scheme for the irreversible inactivation of the title zinc metalloenzyme is proposed.

In connection with a program of organosulphur chemistry and of synthesis of specific metalloenzyme inactivators we have focused our attention on new sulphur derivatives, the β-sulphinamoyl esters $ArNR^1SOC(R^2R^3)COOR$.

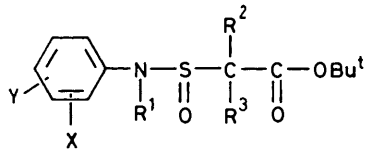
Although many examples of sulphur-containing inactivators have been reported,[1] the biological role of sulphinamoyl compounds has not been mentioned in contrast to the well known sulphonamide series. One reason probably lies in the low number of functionalised sulphinamides. β-Hydroxysulphinamides are the most representative compounds; they have been synthesised as precursors of olefins and ketones by reaction of α-lithiosulphinamide derivatives with aldehydes or ketones.[2] The β-sulphinamoyl ketones or aldehydes were reported only as possible intermediate species but could not be synthesised by oxidation of β-hydroxysulphinamides. Their instability was attributed to their very easy conversion into ketones. We found that the β-sulphinamoyl esters are much more stable and can be isolated in good yield by condensation of lithium or zinc ester enolates with sulphinylamines.[3]

t-Butyl *N*-(2-hydroxyphenyl)sulphinamoylacetate (**1**) constitutes,[4] to our knowledge, the first example of a suicide inactivator of $NAD(P)^+$-dependent dehydrogenase. It inhibits specifically the cinnamoyl alcohol dehydrogenase ($NADP^+$)-(EC 1.1.1.—) (CADH) a zinc metalloenzyme involved in the biosynthesis of lignins[4,5] and *in vivo* leads to a significant decrease of lignification fluxes on forage plants.[4,6] No perturbation of other plant dehydrogenases was detected.

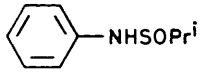
Assuming that the active site of CADH is mainly represented by the zinc ion and that a water molecule is bonded to this ion, as in most zinc dehydrogenases,[7] we examined the kinetics and mechanism of hydrolysis of the t-butyl *N*-arylsulphinamoyl acetates (**1**) and (**2**).

In a preliminary report,[8] we proposed that the sulphinamoyl esters, sulphine precursors, are a new class of irreversible inactivators. We now extend this study to variously substituted compounds in order to determine more accurately the mechanism of hydrolysis for this class of inhibitors.

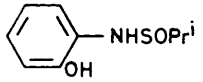
A possible scheme for the irreversible inactivation of CADH is discussed in the light of these kinetic results and of recently reported zinc complexation properties[9,10] of these compounds.



	X	Y	R^1	R^2,R^3
(**1**)	2-OH	H	H	H
(**1'**)	2-OH	H	H	D
(**2**)	3-OH	H	H	H
(**3**)	$4-NMe_2$	H	H	H
(**4**)	4-OMe	H	H	H
(**5**)	4-Cl	H	H	H
(**6**)	3-Cl	H	H	H
(**7**)	3-Cl	5-Cl	H	H
(**8**)	4-COMe	H	H	H
(**9**)	$4-NO_2$	H	H	H
(**9'**)	$4-NO_2$	H	H	D
(**10**)	2-OH	H	H	Me
(**11**)	H	H	Me	H



(**12**)



(**13**)

Experimental

Materials.—Sulphinamoyl esters (**1**)—(**7**), (**9**), and (**10**) were synthesised by condensation of a bromozincate ester enolate with the corresponding sulphinylamine. Compounds (**8**) and (**11**) were obtained by the action of the corresponding aniline on t-butyl chlorosulphinylacetate in the presence of triethylamine at 0 °C and were fully characterised.†

† The synthesis of these compounds will be published elsewhere.

The synthesis and characterisation of compound (**12**) is reported in ref. 9.

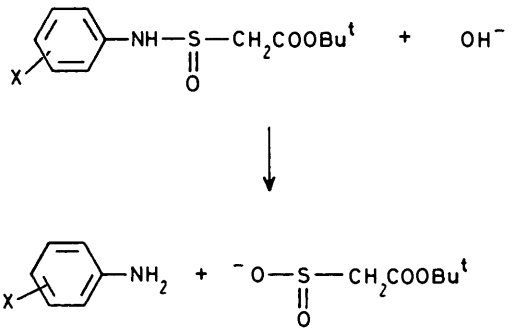
Synthesis of compound (**13**). Pr^iMgCl was obtained by adding excess of isopropyl chloride (80 mmol) to a stirred suspension of magnesium chips (1.6 g, 66 mmol) in dry ether (40 cm^3). 2-Hydroxy-*N*-sulphinylaniline (3 g, 19 mmol) in dry ether (20 cm^3) was then slowly added. A rapid reaction occurred. The mixture was then stirred for 5 min, hydrolysed at 0 °C with 5% aqueous ammonium chloride, and extracted with ether. The solvent was removed and the solid recrystallised from ether–light petroleum (2.4 g, 65%), m.p. 78 °C; $\delta_H(CDCl_3)$ 6.8—7.3 (m, 5 H), 6.5 (s, 1 H), 3.0 (septet, 1 H), 1.4 (d, 3 H), and 1.3 (d, 3 H); $\nu_{max.}(CHCl_3)$ 3 600, 3 400 (OH), 3 300, 3 280 (NH), 1 075 (S=O), and 870 cm^{-1} (S–N).

Synthesis of deuteriated compounds (**1′**) *and* (**9′**). These were obtained by a procedure derived from that described by Starks.[11] To sodium hydroxide (20 mg) in D_2O (5 ml) was added under stirring and inert atmosphere (argon) a solution of cetyltrimethylammonium bromide (CTAB) (15 mg) in D_2O (8 ml). After 5 min a solution of (**1**) or (**9**) (200 mg) in ether [10 ml for (**1**)] or 1:1 ether and THF [10 ml for (**9**)] was added under argon and the mixture was stirred for 1 h. After decantation the aqueous phase was washed four times with ether or ether–THF (10 ml). The organic layer was evaporated. Products (**1′**) and (**9′**) were obtained pure (t.l.c.) after successive washing with small portions of ether to eliminate the anilines formed. Yields were poor [50 mg, 25% for (**1′**), 19 mg, 10% for (**9′**)] because of the degradation of products (**1**) and (**9**). Compound (**1′**) had m.p. 120 °C, $\delta_H(CDCl_3)$ 7.2—6.8 (m, 6 H) and 1.5 (s, 9 H); $\nu_{max.}(CDCl_3)$ 3 400 (OH), 3 250—3 300 (NH), 1 723 (C=O), and 1 070 cm^{-1} (S=O). Compound (**9′**) had m.p. 128 °C, $\delta_H(CDCl_3)$ 8.2, 7.1 (AA′BB′, ^3J 9 Hz, 2 H), 8.1 (s, 1 H), and 1.5 (s, 9H); $\nu_{max.}(CDCl_3)$ 3 280 (NH), 1 720 (C=O), and 1 080 cm^{-1} (S=O).

Kinetics Measurements.—Kinetic measurements were carried out for compounds (**1**), (**2**), and (**10**) with Cary model 15 and Unican model SP 1800 spectrophotometers fitted with an SP 1805 program controller and a thermostatted multiple cell compartment. For the other compounds a Lambda 7 Perkin-Elmer spectrometer equipped with a unit for treatment of the results was used. Pseudo-first-order kinetics were obtained for all compounds up to 95% reaction. A least-square Guggenheim program[12] was used for determining pseudo-first-order constants. pH Measurements were carried out with a Beckman Research pH-meter.

Results

Determination of the Products of the Basic Hydrolysis.—Extensive analysis of final products of the hydrolysis of sulphinamoyl esters was carried out using compounds (**1**) and (**2**). After completing their basic hydrolysis the mixture was extracted with dichloromethane. The product obtained was identified by g.l.c. as the 2- or 3-aminophenol. Analysis of the crude reaction mixture by reverse t.l.c. chromatography with bonded C_{18} silica (35:65 methanol–NaOH, H_2O; pH 10.5) revealed a polar product (R_F 0.85) and the corresponding aniline. The polar product was isolated by h.p.l.c. under the same conditions. Mass [*m/z* 101 $(CO_2Bu^t)^+$ and 64 $(SO_2)^{+\bullet}$] and ^1H n.m.r. spectra [$\delta(CDCl_3)$ 1.45 (9 H, s, t-butyl) and 3.25 (2 H, s, CH_2)] were in good agreement with the structure ^-O–SO–CH_2COOBu^t. In acidic and neutral media, this compound liberates sulphur dioxide and t-butyl acetate. We also verified that the u.v. spectrum of the final mixture from the basic hydrolysis of (**1**) and (**2**) is identical to that of an equimolar mixture of the sodium t-butoxycarbonylmethanesulphinate and the corresponding aminophenol. The same polar product was identified by reverse t.l.c. after basic hydrolysis of compounds (**9**) and (**11**) under the conditions described above.



Kinetics.—The kinetics of hydrolysis of the t-butyl *N*-arylsulphinamoyl acetates (**1**) and (**2**) were studied in detail. They were followed spectrophotometrically by recording the decrease in absorption at 300 nm, resulting from the disappearance of the substrate. The presence of an isosbestic point [284 nm for (**1**) and 273 nm for (**2**)] indicates that there is no build-up of any intermediate species.

pH Dependence.—The basic hydrolysis of (**1**) and (**2**) was studied in the pH range 8.2—12.8 using Tris, borax, and sodium hydrogen carbonate buffers (pH 8.2—10.5) and sodium hydroxide solutions (pH 10.8—12.8). For all the studied buffers general base catalysis was observed and the curves shown in Figure 1 [$\log k'_o$ = f(pH)] were obtained from rate constants extrapolated to zero buffer concentrations $k_{obs} = k'_o + k_g[Buffer]_{total}$, or from those measured in NaOH solutions (Table 1).

For both compounds the profile is sigmoidal. A straight line (pH < 9.2) with a slope of 1.07 for (**1**) and 0.94 for (**2**) was obtained, then a pronounced inflexion and a second straight line (pH > 10.3) with a slope of 1.2 and 1.04 for (**1**) and (**2**) respectively. This profile suggests an ionisation of these compounds and gives kinetic pK_a values of 9.25 for (**1**) and 9.39 for (**2**). These are in good agreement with the spectrophotometric pK_a values 9.10 (**1**) and 9.43 (**2**) calculated from the initial optical density measurements at various pH values. This ionisation can undoubtedly be assigned to the phenol moiety. In fact we measured the pK_a values of deprotonation for the sulphinamoyl nitrogen atom and the hydroxy group, using two model compounds: the *N*-phenylsulphinamide (**12**) and its *N*-(2-hydroxyphenyl) analogue (**13**). For compound (**12**) we found pK_a > 14 while for (**13**) pK_a 9.0 (PhOH).

General Catalysis. Deuterium Oxide Solvent Isotope Effects. Thermodynamic Functions of Activation.—Values of the constants of general base catalysis k_B were obtained from the plots of k_g *versus* the fraction of base, $k_g = (k_B - k_{BH})[B]/[Buffer]_{tot} + k_{BH}$ where $k_{BH} = 0$, and the Brönsted plots were drawn for (**1**) and (**2**) (Figure 2, Table 2). In both cases a straight line was obtained; the point corresponding to the hydroxide ion lies on the line.

Deuterium oxide solvent isotope effects measured in 9.6 × 10^{-3}M-NaOH and 9.6 × 10^{-3}M-NaOD solutions are, respectively for (**1**) and (**2**), k_{OH}/k_{OD} 2.10 and 2.27. No primary isotope effect was observed by comparing the rate constants for (**1**) and (**1′**) in 9.6 × 10^{-3}M-NaOH solutions at 293 K indicating that the deprotonation of the methylene group is not rate determining. The effect of temperature between 228 and 313 K for [OH^-] 5 × 10^{-4}M leads to the following energy and entropy of activation: E_a 43.9 kJ mol^{-1}, $\Delta S^{\ddagger}$ −155 J mol^{-1} K^{-1} for (**1**); E_a 41.8 kJ mol^{-1}, $\Delta S^{\ddagger}$ −167 J mol^{-1} K^{-1} for (**2**).

Substituent Effects.—(*a*) The basic hydrolysis of various sulphinamoyl esters $XC_6H_4NHSOCH_2CO_2Bu^t$ was monitored spectrophotometrically at the appropriate wavelengths and at pH 9.30 and 293 K. As for compounds (**1**) and (**2**) general base catalysis was observed except for the 4-NO_2 derivative (**9**). The first- and second-order rate constants and the electronic parameters σ and σ$^-$ are given in Table 3. When σ parameters are used, the Hammett plot (Figure 3) exhibits a straight line for compounds (**2**)—(**6**) (ρ 0.28) bearing donor or slightly withdrawing substituents. Compounds (**7**)—(**9**) having stronger electron-withdrawing groups deviate strongly and non-linearly from this correlation. Nevertheless when σ$^-$ parameters are used these latter compounds can be fairly located on a second straight line (ρ 3.20). The activation entropy of compound (**9**) [t-butyl *N*-(4-nitrophenyl)sulphinamoylacetate] was determined. It has a positive value, $\Delta S^{\ddagger}$ +23.3 J mol^{-1} K^{-1}.

As for compound (**1**) no primary isotope effect was found from the rate constants of (**9**) and (**9′**) measured at pH 8.25 and *T* 293 K.

(*b*) The effect of the substitution on the carbon atom in the α position of the SO group was also studied. The hydrolysis of

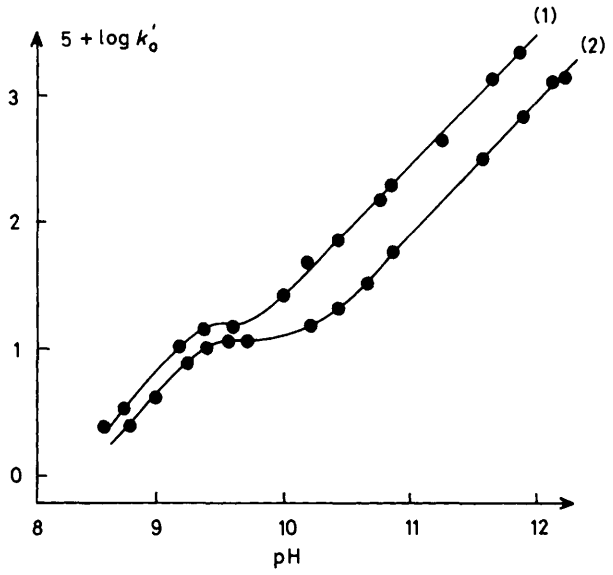


Figure 1. Plot of the logarithm of rate constants k'_0 extrapolated to zero buffer concentration *versus* pH (μ 1.0 KCl, *T* 293 K) for (**1**) and (**2**)

Table 1. Values of rate constants $10^5k'_0$ extrapolated to zero buffer concentration at various pH values for (**1**) and (**2**) [μ 1.0 (KCl), *T* 293 K]

pH		8.40	8.58	8.60	8.81	8.85	8.87	9.00	9.22
$10^5k'_0$/s^{-1}	(**1**)	2.0	3.9	4.0	5.2	5.7			13
	(**2**)			2.2		5.0	4.0	4.4	
pH		9.25	9.41	9.44	9.56	9.65	9.70	10.04	10.15
$10^5k'_0$/s^{-1}	(**1**)		16			17		40	70
	(**2**)	6.9		9.8	9.4		10		18
pH		10.35	10.65	10.85	11.32	11.56	11.70	11.85	12.15
$10^5k'_0$/s^{-1}	(**1**)	75		260	470		1 500	2 200	
	(**2**)	24	34	79		420		910	1 580

Table 2. Values of the logarithms of the general base catalysis constants for the various buffers for sodium hydroxide solutions [μ 1.0 (KCl), *T* 293 K]

Buffers	Tris	Borax	Hydrogen carbonate	OH^-
pK_{buffer}	8.07	8.95	10.09	15.75
$\log k_B$ (**1**)	−2.51	−2.58	−1.47	0.92
$\log k_B$ (**2**)	−2.89	−2.82	−1.79	0.58

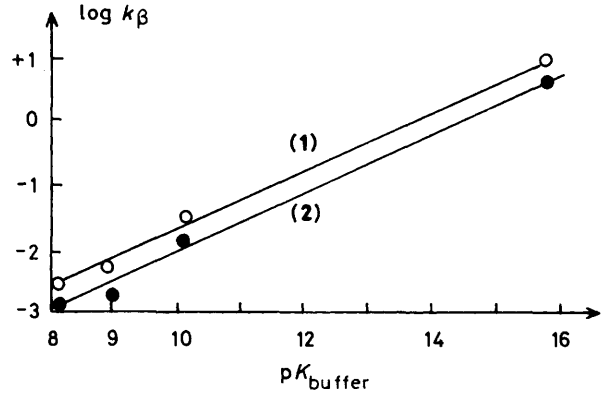


Figure 2. Brönsted plot for the general base catalysis of compounds (**1**) and (**2**) [μ 1.0 (KCl), *T* 293 K]

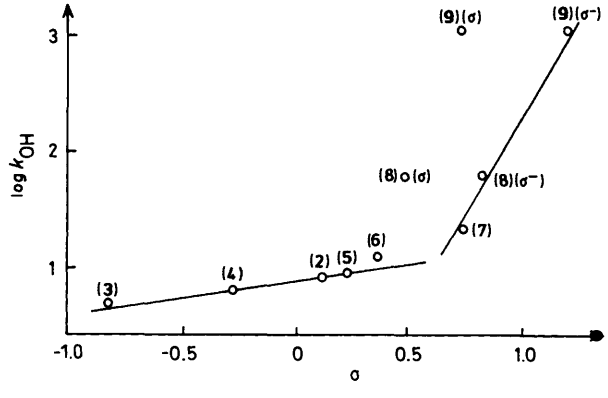
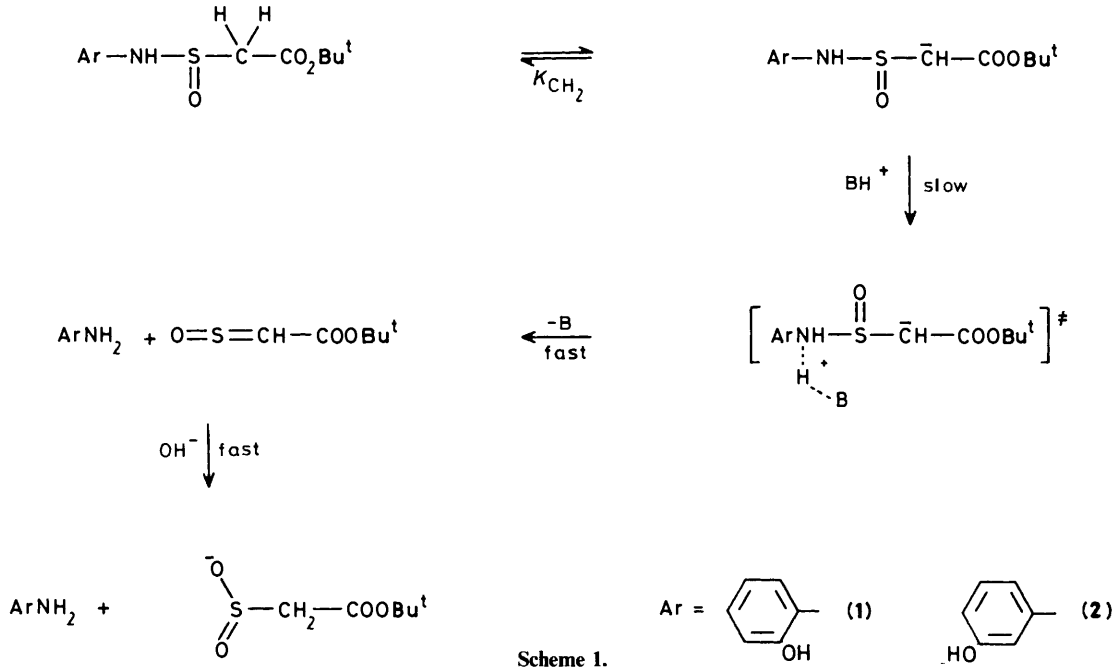


Figure 3. Hammett plot

Table 3. Values of the first-order (k'_0 extrapolated to zero buffer concentration) and second-order (k_{OH}) rate constants: *T* 293 K, μ 1.0 (KCl), pH 9.30 (borax buffer)

Compounds	(**2**)	(**3**)	(**4**)	(**5**)	(**6**)	(**7**)	(**8**)	(**9**)
10^4 k'_0/s^{-1}	0.85	0.95	1.26	1.77	2.25	4.09[a]	11	200
k_{OH}/dm^3 mol^{-1} s^{-1}	5.80	4.77	6.33	8.89	11.30	20.50[a]	55.20	1 005
σ	+0.12	−0.83	−0.27	+0.23	+0.37	+0.74	+0.50	+0.78
(σ$^-$)							(+0.85)	(+1.25)

[a] Measured in water–ethanol (90:10) medium; the values take in account the solvent effect on pH.



Scheme 1.

the dimethyl-substituted compound (**10**) is very slow, and the kinetics were recorded at 323 K. In addition no general catalysis was observed for compound (**10**) the opposite of the case for the unsubstituted compounds (**1**)—(**8**). The deuterium hydroxide isotope effect was measured for $[OH^-] = [OD^-] = 10^{-2}$M. The results for compound (**10**) give k_{OH} 2.28 × 10^{-1}; k_{OD} 2.72 × 10^{-1} dm^3 mol^{-1} s^{-1}; k_{OH}/k_{OD} 0.84.

Discussion

Mechanism of Basic Hydrolysis of Compounds (**1**) *and* (**2**). *Intervention of a Sulphine Intermediate.*—The kinetic results for compounds (**1**) and (**2**) suggest that the same pathway occurs for their basic hydrolysis; no intramolecular effect is present for the *o*-hydroxy compound (**1**) as reported for the hydrolysis of the corresponding *N*-(2-hydroxyphenyl)carbamates.[13]

We propose a mechanism for rate-determining elimination of aromatic amine which takes place on the conjugate base of the substrate resulting from fast deprotonation of the methylene-activated carbon atom.* This elimination assisted by a general acid leads to a sulphine intermediate O=S=CHCOOBu^t; although Hegarty succeeded in trapping an isocyanate intermediate in the E_1cB mechanism for hydrolysis of carbamates,[13] our attempts to trap the proposed sulphine intermediate in this general-base catalysed process were unsuccessful. For instance the dienophile properties of sulphines[14] are not easily transported to an aqueous medium. The intermediate sulphine is rapidly hydrolysed to the final product which was indentified as the t-butoxycarbonylmethanesulphinate ion (Scheme 1).

The observed general base catalysis is thus the product of specific base catalysis and general acid catalysis. The rate equation is (1). The ionisation constant of the activated methylene K_{CH_2} is too weak to be measured kinetically and spectrophotometrically. Thus $K_{CH_2} \ll [H^+]$ and equations (**2**)—(**5**) apply where

$$k_{obs}[S_t] = k_{H_2O}[H_2O]\frac{K_{CH_2}}{K_W}[OH^-][S_t] + k_{BH^+}\frac{K_{CH_2}}{K_B}[B][S_t] \quad (2)$$

$$k_{obs}[S_t] = k_{OH}[OH^-][S_t] + k_B[B][S_t] \quad (3)$$

$$k_{OH} = \frac{k_{H_2O}K_{CH_2}}{K_W}[H_2O] \quad (4)$$

$$k_B = \frac{k_{BH^+}K_{CH_2}}{K_B} \quad (5)$$

k_{H_2O} is the constant of general acid catalysis by water, K_W the ionisation constant of water, k_{BH^+} the constant of general acid catalysis by the buffer, K_B the acidity constant of the buffer. [B] and [S_t] are respectively the concentration of the basic form of the buffer and the total substrate concentration.

Depending on the pH range the phenol moiety may be ionised (K_1) for (**1**) and (**2**) and the reaction takes place on both species. Then equation (6) holds.

$$k_{obs} = k_{OH}[OH^-]\frac{[H^+]}{K_1 + [H^+]} + k'_{OH}[OH^-]\frac{K_1}{K_1 + [H^+]} + k_B[B]\frac{[H^+]}{K_1 + [H^+]} + k'_B[B]\frac{K_1}{K_1 + [H^+]} \quad (6)$$

The k values refer to the reactivity of the phenol form while the k' values are assigned to the phenolate.

The absence of primary isotope effect using the dideuteriated compound (**1'**) indicates that the abstraction of such a proton occurs in a rapid step and thus is in agreement with the proposed mechanism.

In this mechanism the water molecule, conjugate acid of hydroxide ion, acts as a general acid in agreement with its position on the Brönsted plot. The negative entropy of

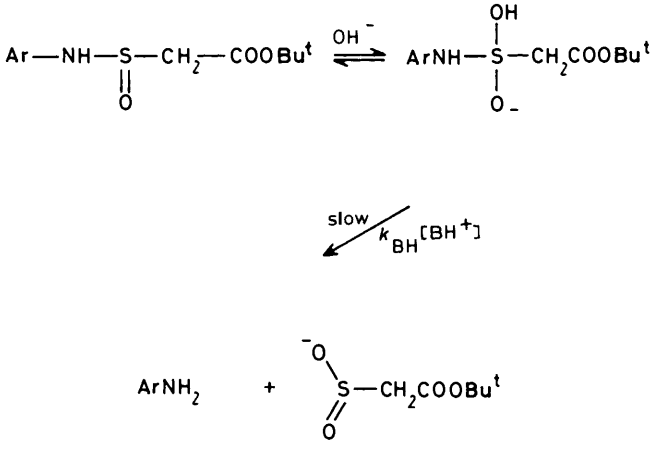
* In a preliminary report[8] we proposed a mechanism where the rate-limiting step was deprotonation of the methylene group. The absence of primary isotope effect rules out this hypothesis.

activation measured for compounds (**1**) and (**2**) agrees with an associative bimolecular mechanism. The value of the solvent isotope effect (k_{H_2O}/k_{D_2O} 2.1—2.3) cannot be used as confirmation of the proposed mechanism. It was measured in sodium hydroxide solutions where expression (7) is found for the rate constant.

$$k'_{OH} = \frac{k'_{H_2O}K_{CH_2}}{K_W}[H_2O] \qquad (7)$$

As mentioned earlier the K_{CH_2} value could not be measured. Hence the value of the solvent isotope effect on this ionisation constant is not known and the k'_{H_2O}/k'_{D_2O} ratio cannot be calculated.

Another mechanism analogous to that reported for formanilides[15] may be considered. It would proceed by slow protonation by a general acid of a tetrahedral intermediate formed from rapid hydroxide ion attack on the sulphinyl sulphur atom of the substrate. After decomposition it would lead to the final products (Scheme 2). The kinetic equation is the same as that for the mechanism in Scheme 1. However it can be ruled out because it is inconsistent with our results for compounds (**1**), (**2**), (**9**), and (**10**).



Scheme 2.

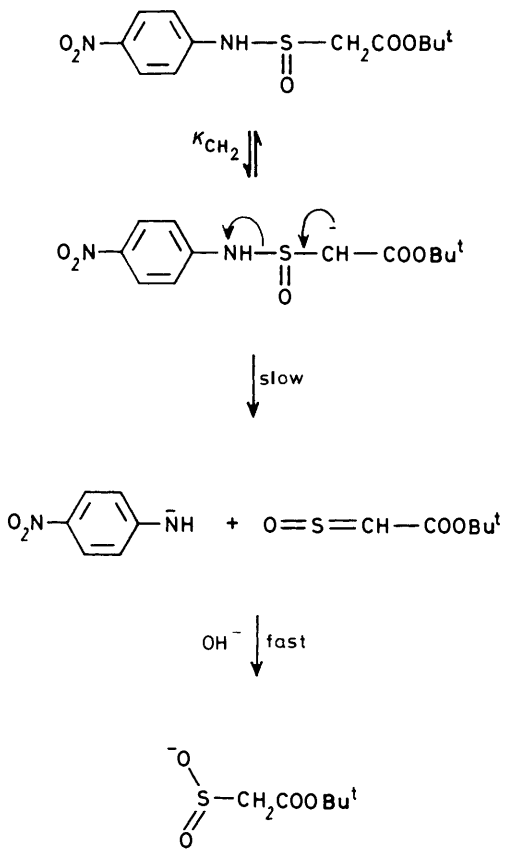
Compound (**10**) where the two methylene hydrogen atoms are replaced by methyl groups cannot be hydrolysed by the mechanism observed for the unsubstituted compounds (**1**) and (**2**), while if the mechanism was analogous to that observed for amides (Scheme 2) not fundamental differences would appear. For compound (**10**) no general catalysis was observed during hydrolysis as offered to compounds (**1**) and (**2**); the solvent isotope effect (k_{H_2O}/k_{D_2O} 0.84) is very different from that observed for (**1**) and (**2**). It is likely that compound (**15**) decomposes *via* a $B_{Ac}2$ mechanism of slow attack of hydroxide ion on the sulphinyl sulphur atom.

Another argument which makes us prefer the mechanism in Scheme 1 to that of Scheme 2 arises from the positive entropy of activation for compound (**9**) compared with the negative one for compounds (**1**) and (**2**). For formanilides decomposing by a pathway analogous to Scheme 2, the entropy of activation for the *p*-nitro derivative remains strongly negative as for all other substituted derivatives.[15]

Hammett Plot and Change of Mechanism.—We have previously mentioned that the Hammett plot (Figure 3) exhibits two straight lines; the first as a function of σ (slope ρ 0.28) concerns compounds with donor or poorly withdrawing groups, while the second as a function of σ^- (slope ρ 3.20) is for compounds (**7**)—(**9**) with strong withdrawing groups.

The value of the slope for the first correlation (ρ 0.28) could result from opposing effects of the donor or slightly withdrawing groups of the aromatic ring on the acidity of the methylene protons (K_{CH_2}) and on the basicity of the nitrogen atom of the leaving group (k_{H_2O}).

For the more electron-withdrawing substituents (**7**)—(**9**) the presence of general catalysis is very significantly attenuated (or negligible for (**9**)] and there is a correlation with σ^-, with an increased slope (ρ 3.20). It indicates that a conjugation interaction exists between the substituent and a negative charge developing on the nitrogen atom in the transition state. In the case of compounds substituted by strongly electron-withdrawing groups, the aromatic amide ion is sufficiently good a leaving group that a general catalysis is unnecessary. For this class of compounds we focused our attention on the extreme 4-nitro derivative (**9**). The arguments developed below indicate an E_1cB mechanism (Scheme 3).



Scheme 3.

As for compound (**1**) no primary isotope effect was observed, using the dideuteriated methylene compound (**9'**).

The temperature effect was measured for four temperatures in the range 283—298 K. For compounds (**2**) and (**5**) which are located on the first Hammett line (ρ 0.28), the respective values of −167 and −138 J mol^{-1} K^{-1} are indicative of an associative mechanism. On the other hand, for the nitro compound (**9**) a positive value (+23.3 J mol^{-1} K^{-1}) is characteristic of monomolecular elimination.[16]

The slope of the Hammett correlation for this compound is analogous to that found for the basic hydrolysis of aryl carbamates ArNHCOOAr by an E_1cB mechanism.[13]

Our results are somewhat different from those recently

reported by Kice[17] concerning the mechanism of reaction of MeOH–MeO^- on the methyl diarylsulphinates. He suggested an $(E_1cB)_{irrev.}$ or E_2 elimination mechanism with slow abstraction of the proton α to the sulphinate group followed by a sulphine formation. He considers that the mechanistic behaviour of these compounds is atypical and deviates from that found by Williams[18] for the analogous sulphonate esters.

For the sulphinamoylacetate series we found that the abstraction of the activated proton occurs in a rapid step. For poor leaving groups an electrophilic assistance is necessary (general acid catalysis) while for better leaving groups it is not useful and an $(E_1cB)_{rev.}$ elimination takes place.

Possible Mechanism of the Action of the Sulphinamoyl Ester and Related Compounds.—As mentioned earlier[4] t-butyl *N*-arylsulphinamoylacetate (**1**) is a selective and effective inactivator of CADH characterised by a pseudo-irreversible inhibition. Enzymatic kinetic data suggest that inactivation occurs before the inactivator is released from enzyme, a fundamental characteristic of suicide inactivation. Strong association between the enzyme and the inactivator presumes a breaking of a bond of the starting compound and the formation of either a covalent bond with an amino acid at the active site of the enzyme, or a very strong metal–inactivator association.

On the basis of the results obtained for basic hydrolysis, the following scheme of inactivation is proposed.

As a first step, the potential inactivator (**1**) is complexed with zinc ion at the active site: this complexation should take place through the oxygen of the suphinyl group and the *o*-hydroxy group, by analogy with the results obtained by modelling the complexation with $ZnBr_2$.[10]

As a second step, as suggested by the results of basic hydrolysis and also by the necessary presence of a CH_2 group α to the sulphinyl group [$ArNHSOC(Me)_2COOBu^t$ does not inactivate CADH at all[4]], a proton of this methylene group is extracted by base (the water molecule being bonded to zinc or a basic atom of an amino acid of the protein chain). Moreover, this deprotonation should be facilitated by the complexation S=O · · · Zn thus reinforcing the polarisation of the sulphinyl bond.

Finally, two species should be generated by the breaking of the N–S bond, 2-aminophenol (inactive) and the sulphine. This sulphine (the unmasked inhibitor) could link to the protein by reaction with a nucleophile of the chain or give, by reaction with a water molecule, the t-butoxycarbonylmethanesulphinate ion able to complex strongly with zinc.

A comparison of the kinetic results, *i.e.* the change in the mechanism of hydrolysis of the sulphinamoyl esters with the biological behaviour of these inactivators, is in progress.

Acknowledgements

This work was supported by the Centre National de la Recherche Scientifique (ARI Interface chimie-biologie) and Université Paul Sabatier (Programme prioritaire). We thank Dr. A. M. Boudet and his co-workers who studied the inactivation features of CADH, Dr. M. Bergon for helpful discussions, and Roussel-Uclaf for a grant for one of us (M. B.).

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Received 14*th November* 1986; *Paper* 6/2199