Reaction of Amine Nucleophiles with a Disulphide (2,2'-Dinitro-5,5'-dithiodibenzoic Acid): Nucleophilic Attack, General Base Catalysis, and the Reverse Reaction

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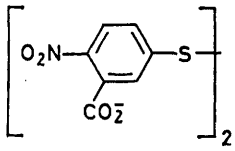
Secondary and primary amines react with a disulphide (Ellman's reagent, 2,2'-dinitro-5,5'-dithiodibenzoic acid) in a reaction which is not base catalysed. The primary amines possess a Brønsted β_{nuc} of +0.45 consistent with only partial bond development between N and S in the transition state. Proton transfer to yield the stable sulphenamide occurs in a fast step after N–S bond formation has occurred. Tertiary amines catalyse the disulphide cleavage and possess a high Brønsted β (+0.8) and trimethylamine has a solvent deuterium isotope effect (k_{Me_3N}/k_{Me_3N} 1.72) consistent with the general base catalysed attack of water. The normally more efficient nucleophilic attack would yield *NNN*-trialkylsulphenamides which, together with the thiolate anion co-product, is in unfavourable equilibrium with the reactants; steric hindrance also contributes to the inefficiency of the nucleophilic mechanism.

NUCLEOPHILIC substitution at dico-ordinate sulphur presents an important problem concerning the timing of the bond forming process compared with leaving group departure. Several important observations have already been reported concerning this problem [1] and it is generally supposed that the process is synchronous and does not involve an intermediate. Probably the most significant reaction of this class is the disulphide cleavage in proteins.[2a]

In attempting to use Ellman's reagent (I) to measure



(I)

the thiol content of polyethyleneimines modified with mercapto groups we observed considerable background cleavage by material containing no thiols and it was found that alkylated polymer was a very efficient catalyst of this process.[3] We are interested in the attack of simple amines on the disulphide link because we require to know the rate constants in order to calibrate the efficiency of the polymeric amine catalysts in this reaction. This investigation is of the release of thiol from Ellman's reagent caused by a series of primary, secondary, and tertiary amines. Early work in the area of nitrogen nucleophile attack on divalent sulphur includes the pioneering work of Foss on native sulphur and later work on other sulphenic acid derivatives [1b-d,k,4] but it is not sufficient to provide us with accurate numerical data for comparison purposes.

EXPERIMENTAL

Materials.—Amines were obtained as the free base or hydrochloride from B.D.H. or Aldrich and were purified by redistillation or recrystallisation; Ellman's reagent was from Aldrich. 2-Nitrobenzenesulphenamide was prepared from the sulphenyl chloride and ammonia; it had m.p. 124—125°(lit.,[2b] 124—125°). Other material was of analytical grade or was redistilled or recrystallised from bench grade products. Deuterium oxide (99.8% D) was from Prochem and water used throughout the investigation was doubly distilled from glass.

Methods.—Kinetics were measured spectrophotometrically *via* the release of thiol and are as described in ref. 3. Precautions were taken [3] to prevent oxidation of the released thiol by air. Measurements of pH were made with either a Pye-Dynacap or a Radiometer PHM-26 instrument calibrated with E.I.L. buffers to ±0.02 pH units. The buffers utilised in the sulphenylation experiments were constituted from the amines employed as nucleophiles. Analysis of the pH profiles was by a BASIC computer program devised by Mr. C. R. Farrar and run by the University of Kent Computer Centre. A Physical and Electronics Laboratories analogue computer model C180 was used in the simulation experiments in conjunction with a Bryans X–Y recorder.

RESULTS

The release of thiol as measured by the absorbance at 410 nm was accurately first order over *ca.* 90% of the total reaction between Ellman's reagent and nucleophile. The amount of thiol released in these experiments was that expected for complete scission of the disulphide bond as determined from the extinction coefficient for the thiol at the pH in question [3] except for the cases mentioned below.

The rate constants for thiol release were linear in total amine concentration and were insensitive to added base indicating that the reaction is only between amine and substrate. Studies over a pH range with methylamine indicated that the basic form of the amine was responsible for the reaction (see Figure 1); bimolecular rate constants

[1] (*a*) E. Ciuffarin and A. Fava, *Progr. Phys. Org. Chem.*, 1968, **6**, 81; (*b*) L. Senatore, E. Ciuffarin, and A. Fava, *J. Amer. Chem. Soc.*, 1970, **92**, 3036; (*c*) L. Senatore, E. Ciuffarin, and L. Sagramore, *J. Chem. Soc.* (*B*), 1971, 2191; (*d*) E. Ciuffarin, L. Senatore, and M. Isola, *ibid.*, p. 2187; (*e*) J. L. Kice, T. E. Rogers, and A. C. Warheit, *J. Amer. Chem. Soc.*, 1974, **96**, 8020; (*f*) C. Brown and D. R. Hogg, *Chem. Comm.*, 1967, 38; (*g*) L. di Nunno, G. Modena, and G. Scorrano, *Ricerca Sci.*, 1966, **36**, 825; (*h*) A. Fava and G. Pajaro, *J. Amer. Chem. Soc.*, 1956, **78**, 5203; (*i*) A. Fava, A. Iliceto, and E. Camara, *ibid.*, 1957, **79**, 833; (*j*) J. L. Kice, *Mech. React. Sulphur Compounds*, 1968, **3**, 91; (*k*) A. J. Parker and N. Kharasch, *Chem. Rev.*, 1959, **59**, 583; (*l*) R. E. Davis, *Survey Progr. Chem.*, 1964, **2**, 189; (*m*) J. L. Kice, *Accounts Chem. Res.*, 1968, **1**, 58; (*n*) J. L. Kice and J. M. Anderson, *J. Org. Chem.*, 1968, **33**, 3331.

[2] (*a*) P. C. Jocelyn, 'Biochemistry of the SH group,' Academic Press, London, 1972; (*b*) J. H. Billman and E. O'Mahony, *J. Amer. Chem. Soc.*, 1939, **61**, 2340.

[3] R. H. Weatherhead, K. A. Stacey, and A. Williams, *J.C.S. Perkin II*, in the press.

[4] (*a*) O. Foss in 'Organosulphur Compounds,' ed. N. Kharasch, Pergamon, Oxford, 1961, vol. 1, ch. 9; (*b*) R. E. Davis, 'Organosulphur Compounds,' ed. N. Karasch, Pergamon, Oxford, 1963, vol. 3, p. 1; (*c*) R. E. Davis and H. F. Nakshbendi, *J. Amer. Chem. Soc.*, 1962, **84**, 2085; (*d*) M. E. Peach, *Canad. J. Chem.*, 1967, **45**, 429.

were derived for the other amines at a constant pH with the reasonable assumption that the base form is acting in these cases also. The results are recorded in the Table. The

It was observed that morpholine and piperazine in its low pK_a form did not release the full amount of thiol as expected from the extinction coefficient at the pH in

Reaction of nucleophiles with Ellman's reagent [a]

	Nucleophile	Concentration range (M) [b]	Base fraction	pK_a	k_1/l mol^{-1} s^{-1}
1	Piperidine	0.2—1.0(5)	0.25	10.90	4.8×10^{-2}
2	Hydrazine	0.1—0.5(4)	0.5	8.46	3.7×10^{-3}
3	Morpholine [g]	0.2—1.0(5)	0.5	8.96	9.3×10^{-4}
4	Piperazine [f]	0.4—1.0(3)	0.5	9.96	8.3×10^{-3}
5	Ethylenediamine	0.2—0.5(4)	0.5	10.32	4.6×10^{-3}
6	EthylenediamineH$^+$ [d]	0.3—0.5(2)	0.5	7.42	2.4×10^{-4}
7	Tris [c]	0.3—0.5(3)	0.5	8.22	$<4 \times 10^{-4}$
8	Ethylamine	0.4—1.0(4)	0.5	10.80	1.3×10^{-2}
9	Ethanolamine	0.4—1.0(4)	0.5	9.8	4.2×10^{-3}
10	Benzylamine	0.4—1.0(4)	0.5	9.40	1.8×10^{-3}
11	Trimethylamine H	0.2—1.0(4)	0.5	9.96	1.24×10^{-3}
	D	0.2—1.0(4)	0.5	(D_2O)	0.72×10^{-3}
12	Triethylenediamine	0.6—1.0(3)	0.5	9.18	3.6×10^{-4}
13	Hydroxide	(18)			1.4 [e]
14	Water	(18)		−1.7	$1.2\ 10^{-4}/55.5 = 2.2 \times 10^{-6}$
15	*N*-Methylmorpholine	0.2—1.0(5)	0.5	7.83	$<1 \times 10^{-5}$
16	β-Alanine	0.2—1.0(5)	0.5	10.3	4.2×10^{-3}
17	Imidazole	0.6—1.0(3)	0.5	7.0	2.5×10^{-4}
18	Dimethylamine	0.2—1.0(5)	0.5	11.1	2.8×10^{-2}
19	Diethylamine	0.3—1.0(5)	0.5	11.24	5.4×10^{-3}
20	Methylamine	0.2—1.0(20)	0.25—0.75	10.94	1.2×10^{-2}
21	*N*-Methylpyrrolidine	0.2—1.0(5)	0.5	10.58	1.3×10^{-3}

[a] Ionic strength maintained at 1.0M with KCl, 35°; concentration range of Ellman's reagent 0.5—1 × 10^{-6}M. [b] Number of points used for the determination of k_1 in parentheses. [c] Trishydroxymethylaminomethane. [d] Monoprotonated form of the diamine. [e] Determined from the intercept at pD 10.52 using pD = ' pH reading ' + 0.37 (A. Williams, *J.C.S. Perkin II*, 1975, 947) and $K_w^D = 10^{-14.81}$ (interpolated from data in R. W. Kingerley and V. K. LaMer, *J. Amer. Chem. Soc.*, 1941, **63**, 3256; K_w^H from V Gold, ' pH-Measurements,' Methuen, London, 1956 ($10^{-13.68}$)]. [f] Piperazine in its low pK_a form (monoprotonated) gave very little release of thiol and no estimates of k_1 are possible. [g] Morpholine gave reduced thiol release (Figure 3) and k_1 is estimated from the slope of k_{obs} *versus* free amine concentration.

intercept of the pseudo-first-order rate constants for a given amine at zero buffer concentration was not negligible and was pH-dependent (and buffer independent); the rate constant followed the rate law (1) and the parameters best

$$k = k_{H_2O} + k_{HO}[OH^-] \quad (1)$$

fitting the data are recorded in the Table. The pH profile for the intercepts is illustrated in Figure 2.

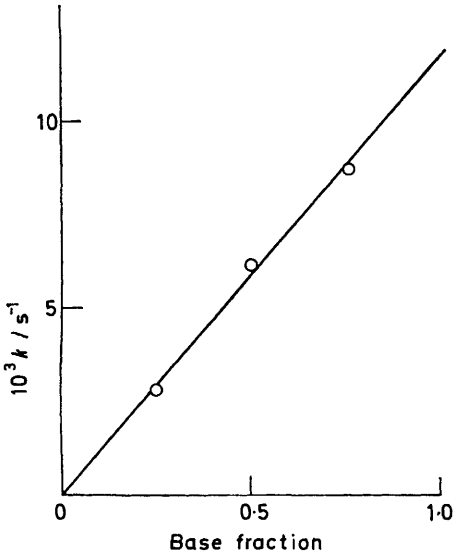


FIGURE 1 Plot of the slope of k_{obs}/[total methylamine] at constant pH *versus* the fraction of base at 35°, with the ionic strength maintained at 1M with KCl. Line is theoretical from the Table

question. Addition of excess of glutathione to the solution when the absorbance at 400 nm had reached a maximum

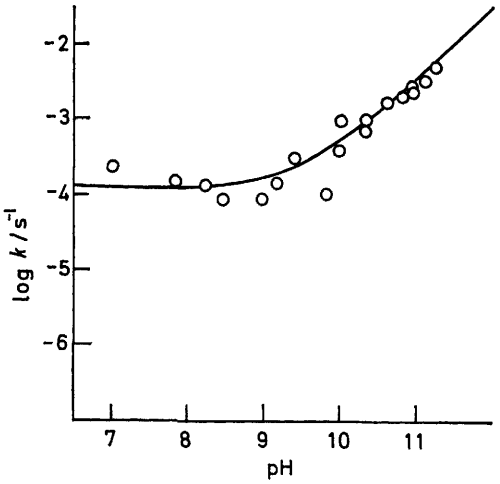
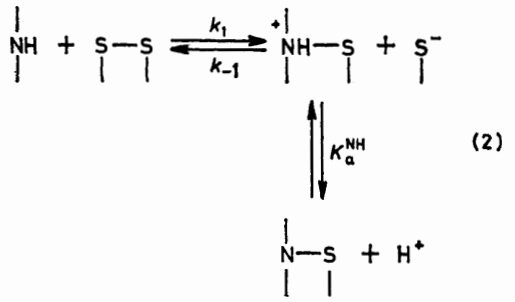


FIGURE 2 Dependence of the intercept at zero buffer concentration on the pH for the rate constant for release of thiol from Ellman's reagent. Ionic strength held at 1M with KCl; line is theoretical from parameters in Table, 35°

caused a further increase to yield the expected absorbance. The simplest kinetic scheme consistent with this result is shown in equation (2) where the formation of product is reversed by attack of thiolate anion on the protonated sulphenamide. The rate law for approach to equilibrium of such a system is complex because the kinetic scheme is equivalent to equation (3) where the forward rate constant is pseudo-first order ([amine]k_1) and the sulphenamide is

produced with an equivalent amount of thiolate anion; the reverse rate constant is given by equation (4) where K_a^{RSH} is the dissociation constant of the species RSH.



(2)

The kinetic scheme of equation (3) may be modelled by an analogue computer network [equation (5)] which indicates that although the kinetics for approach to equilibrium are complex they would be indistinguishable in a

$$A \underset{k_R}{\overset{k_F}{\rightleftharpoons}} 2B \qquad (3)$$

$$k_R = k_{-1} \cdot \frac{1}{(1 + a_H/K_a^{NH})} \cdot \frac{1}{(1 + K_a^{RSH}/a_H)} \qquad (4)$$

real experiment from first order even though at equilibrium only a fraction of A may be converted into B. With settings of k_R at 0.05 s^{-1} and k_F varying from 1.00 to 0.025 s^{-1} first-order tracings were obtained with a slight initial deviation; a linear correlation exists between the 'observed' rate constant for the approach to equilibrium of the system and k_F. The slope of this linear correlation is unity; thus for the real reaction where the equilibrium may lie mid-way between reactants and products the slope of a plot of k_{obs} *versus* the concentration of the free amine is the bimolecular rate constant for the attack of the nucleophile on the disulphide (k_1). As the percentage yield of B increases the pseudo-first-order rate constant becomes closer to k_F; that is, in the real experiment the rate constant becomes strictly proportional to the free amine concentration within the limits of experimental error.

In the case of the monoprotonated piperazine the reverse reaction (k_R) is sufficiently large that very little yield is observed and it is not possible to obtain an accurate value for the forward rate constant (k_1). Morpholine buffers give yields from *ca.* 20 to 80% of theoretical and a plot of k_{obs} *versus* free base is linear with a slight intercept at zero buffer concentration as predicted by the analogue computer; the slope is taken to be k_1 (see Table).

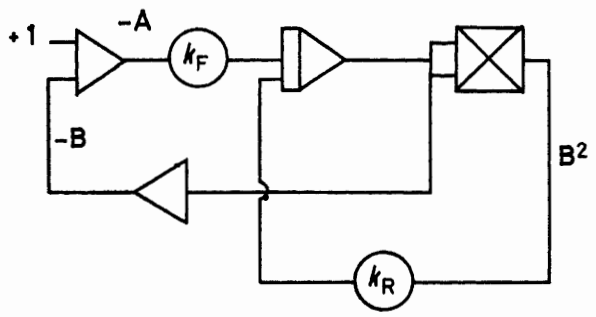
Further complications such as reaction of sulphenamide product with remaining disulphide are not very likely because of the low concentrations of both disulphide and sulphenamide.

Deuterium oxide solvent isotope effects were measured for the trimethylamine system; the intercept measurement for this gives a value for k_{OD^-} and the slope of the pseudo-first-order rate constants *versus* amine concentration gives the value for k_{Me_3N} in deuterium oxide.

DISCUSSION

The kinetic data are consistent with rate-limiting attack of neutral amine on Ellman's reagent and the absence of general base catalysed amine attack indicates that proton transfer occurs after the initial reaction [equation (2)]. The release of one thiol per mole of Ellman's reagent consumed is good evidence that sulphenylation has taken place.

The observation that the full amount of thiol is not produced for morpholine (Figure 3) or piperazine in its low pK_a form indicates that a back reaction is occurring commensurate in rate with the forward. That an equilibrium is being set up [equation (2)] is confirmed by the release of a quantitative amount of extra thiol on addition of glutathione which provides an alternative pathway to product. The existence of back reaction is presumably because the thiolate anion product is such a powerful nucleophile and is sufficiently active to overcome the effect of its low concentration. For amine nucleophiles of relatively high basicity full thiol release is observed and this is probably because the relative leaving ability of the amine is poor compared with that of more weakly basic amines. The observation that full thiolate release is not observed with amines at low pH is also consistent with the scheme [equation (2)]. The pH dependence of the reverse rate constant is theoretically bell-shaped due to ionisation of the thiol and sulphenamide substrate [equation (4)]. The pK_a values of the species involved are all less than the pH values employed in this study so that the reverse rate constant (k_R) will always decrease with decreasing pH.



(5)

It is impossible at this stage to estimate k_{-1} because the reverse rate involves the ionisation of *N*-protonated sulphenamide and we can only place an upper limit on the pK_a of this species. Spectrophotometric and potentiometric titration of 2-nitrobenzenesulphenamide fail to reveal evidence for an ionisation above pH 2 and this result is in agreement with reasonable expectation; it was found that benzaldehyde ethyl thioacetal [5a]

[5] O. Foss, *Acta Chem. Scand.*, 1947, **1**, 307.

exchanges protons some 10^6-fold faster at the α-carbon atom than does the corresponding oxygen analogue in solution with potassium alkoxide. Thus the pK_a of the

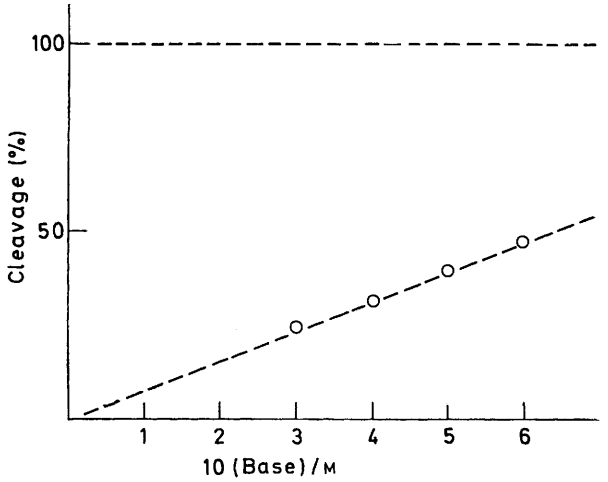


FIGURE 3 Percentage cleavage of Ellman's reagent as a function of morpholine free base concentration; values are for full attainment of equilibrium. In this experiment the total molarity of morpholine is held constant and the pH varied. Allowance is made for the variation with pH of the extinction coefficient of the Ellman cleavage. The values of pH are 8.47, 8.63, 8.84, and 9.09

sulphenamide NH^+ is reasonably expected to be *ca.* 6 units smaller than that of the corresponding hydroxylamine derivative.

The attack of nucleophiles on *N*-protonated sulphenamides is supported by data in the literature; an analytical procedure for sulphenamides involves their

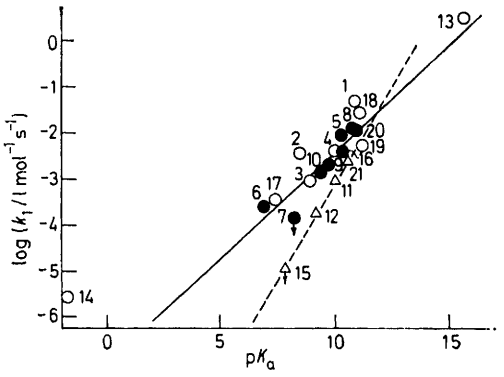
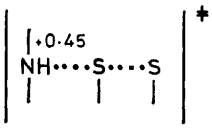


FIGURE 4 Dependence of the rate constant (k_1) on the pK_a of the conjugate acid of the nucleophile for the release of thiol from Ellman's reagent. Ionic strength is held at 1M with KCl, 35°; the lines have the slopes: dotted, 0.8; full, 0.45; filled circles are primary amines, triangles are tertiary amines, and open circles are mainly secondary amines. The numbering system and data are from the Table

reaction with thiosulphate in acid [5a] and sulphenamides are easily hydrolysed by hydrochloric acid.[6]

Since the aim of this paper is to investigate the nucleophilic efficiency of amines in their attack on disulphides we defer further work on this interesting equilibrium to a later date.

Primary amine attack on the disulphide (Figure 4) possesses a Brønsted type correlation with the pK_a of the ammonium ion (β_{nuc} 0.45). Six-membered ring secondary amines, imidazole, and other secondary amines do not deviate markedly from the primary amine correlation. Previous work [1] has indicated that attack of nucleophiles on divalent sulphur is essentially a concerted one and the low β_{nuc} found here is consistent with this. Since we do not yet know how the equilibrium constant for amine attack varies with pK_a of the amine it is not possible to indicate the ' effective charge ' [8a] on the nitrogen in the transition state; if the ionisation of the ammonium ions is taken as the calibrating equilibrium then the nitrogen has +0.45 units of ' effective charge ' (II). It is possible that the charge is less than this because the transfer of other acyl functions (carbamate, carboxy, carbonate, sulphonate) are known to be *more* sensitive to substituents on the acceptor group than is the transfer of the proton to that acceptor.[7,8b-d] This is tantamount to saying that the



(II)

acyl function induces positive charge on the acceptor atom. It is thought that the protonated sulphenamide has a much lower pK_a than the corresponding ammonium ion which suggests that sulphenyl group transfer is more sensitive than proton transfer to change in substituent. We are aware, of course, that the acyl groups cited above are not very good structural models for the sulphenyl group but they (in common with the latter) have an electron-withdrawing effect.

The work of Brown and Hogg [1f] and Kice and Anderson [1n] indicates that there is very little change in charge at the sulphur undergoing nucleophilic attack in sulphenyl group transfer. Ciuffarin and Fava [1a] find that there is evidence for a negative charge on the sulphur in the transition state for exchange of $ArS-SO_3^-$ with SO_3^{2-} (ρ +0.85); comparison with the ρ value for the ionisation of phenols (−2.2) [8e] and thiophenols (−2.2) [8e] indicates that the substituents on the aromatic group ' see ' −0.39 effective charge on the electrophilic sulphur in the transition state. Since the total charge carried by the transition state is high (−3 units) it may be that the charge ' seen ' by the substituents does not reside on the electrophilic atom. In the present case we can say that *if* the ' effective charges ' were additive then there should be an increase of −0.45 units on the leaving

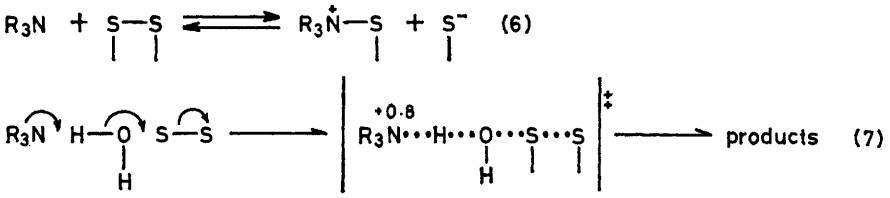
[6] N. Kharasch, S. J. Potempa, and H. L. Wehrmeister, *Chem. Rev.*, 1946, **39**, 269.

[7] (*a*) T. Deacon, A. Steltner, and A. Williams, *J.C.S. Perkin II*, 1975, 1778; (*b*) C. R. Farrar and A. Williams, *J. Amer. Chem. Soc.*, 1977, **99**, 1912; (*c*) T. Deacon, B. Sikkel, C. R. Farrar, and A. Williams, unpublished work.

[8] (*a*) W. P. Jencks, *Cold Spring Harbor Symposia on Quantitative Biology*, 1971, **36**, 1; (*b*) H. Al-Rawi and A. Williams, *J. Amer. Chem. Soc.*, 1977, **99**, 2671; (*c*) J. Gerstein and W. P. Jencks, *ibid.*, 1964, **86**, 4655; (*d*) C. K. Sauers, W. P. Jencks, and S. Groh, *ibid.*, 1975, **97**, 5546; (*e*) G. B. Barlin and D. D. Perrin, *Quart. Rev.*, 1966, **20**, 75.

sulphur atom. This would imply a large leaving group effect which is borne out by studies on the leaving group ability from sulphenic acid derivatives.[1b,c]

Hydrazine, a typical ' α-nucleophile ' shows very little deviation from the primary amine line; this small deviation is consistent with the low β_{nuc} value in accord with the relationship between the α-effect and β discovered by Dixon and Bruice[9a] and predicted by Hudson.[9b]



(6) (7)

General Base Catalysis by Tertiary Amines.—Reference to Figure 4 indicates that tertiary amines catalyse thiol release from Ellman's reagent and that the β value is greater than that for primary amines. Moreover the tertiary amines become less efficient than primary amines for pK_a values in the range 7—9. It is unlikely that nucleophilic attack at sulphur is responsible as we should expect the tertiary amines to possess a similar β value to that for primary amines. The reactivity for a hindered primary amine (trishydroxymethylaminomethane) is low due to steric hindrance and it is therefore probable that tertiary amines will be much less reactive than primary ones on account of their greater steric requirements. The deuterium oxide solvent isotope effect of 1.7 (Table) is good evidence that a proton transfer is involved in the rate limiting step;[10] the value is to the lower end of the range expected.[10] We propose that tertiary amines are involved in general base catalysed water attack; the usually more efficient nucleophilic pathway (in the pK_a range 7—9) must be inhibited otherwise it would carry the major part of the reaction flux and the general base process would not be expressed. Apart from the steric effect mentioned above a further inhibition mechanism is probably that the *NNN*-trialkylsulphenamides produced by attack of tertiary amines on the disulphide are very unstable and react with the strongly nucleophilic thiolate anion to regenerate starting material [equation (6)]; essentially similar behaviour has been observed with tertiary amines on sultones.[7] We have already shown that even in the case of primary and secondary amines the full reaction may not occur because although the protonated sulphenamide may not be present in very high concentration the amine leaving group is exceptionally effective and the nucleophile is powerful. Thus the back reaction effectively inhibits nucleophilic attack by tertiary amines. Using the arguments applied to the attack of primary amines the effective charge on the nitrogen in the transition state is +0.8 for the reaction calibrated by the ionisation of the ammonium species (7).

The water and hydroxide parameters (Figure 4) seem to fit the nucleophilic line rather than the general base correlation and it is probable that these species act as nucleophiles. The inverse deuterium isotope effect observed for the hydroxide parameter is consistent with this conclusion.

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[9] (*a*) J. E. Dixon and T. C. Bruice, *J. Amer. Chem. Soc.*, 1962, **84**, 595; (*b*) R. F. Hudson in ' Chemical Reactivity and Reaction Paths,' ed. G. Klopman, Wiley, New York, 1974, p. 167.

[10] (*a*) M. L. Bender, E. J. Pollock, and M. C. Neveu, *J. Amer. Chem. Soc.*, 1962, **84**, 595; (*b*) S. L. Johnson, *Adv. Phys. Org. Chem.*, 1967, **5**, 237.