IODOALKANESULPHONATES AS THIOL REAGENTS*

By M. A. JERMYN†

Iodoalkanesulphonates offer possibilities as substituents for cysteinyl side-chains of reduced proteins by combining the advantages of reduction methods, which modify only cystine residues,[1] with those of performic acid oxidation, the stability of the sulphonic acid group to hydrolysis or further oxidation. There is a lack of any information about this alkylation reaction other than the physiological inertness of iodomethanesulphonates. The use of solutions of sodium iodomethanesulphonate (Methiodal Sodium; U.S.P.) as a contrast medium in the radiography of the urinary tract with little toxic effect suggests that the reactivity of this compound with the thiol groups of protein must be low.

The comparative reactivity of three iodoalkanesulphonates with *p*-nitrothiophenol has now been determined, and is contrasted with the reactivity of iodoacetamide (Table 1). This system has been chosen because the reaction is easily followed

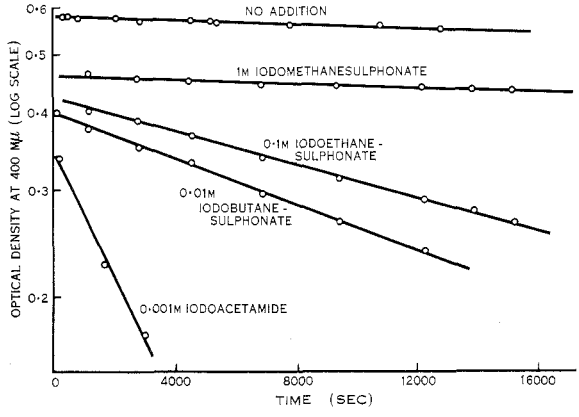

Fig. 1.—Disappearance of *c.* 5×10^{-5}M *p*-nitrothiophenol in 0·4M triethylamine–hydrochloric acid buffer at pH 10·4 and 20° in the presence of various reactants.

and self-indicating (Fig. 1), and the thiol will be present entirely in the reactive, negatively charged form in the pH range studied, so that the effect of ionization equilibria may be neglected. Inspection of Table 1 shows that pH in fact has little effect on the rate constants over the range 9·2–11·0. The differences between the rate constants for different reactants seem to depend essentially on relative reactivities.

The rate of reaction for iodoalkanesulphonates is increasingly depressed below that for the neutral compound with the closer approach of iodo and sulphonate groups, the depression amounting to virtually complete inhibition for iodomethanesulphonate. Nevertheless, 4-iodobutanesulphonate could be used to modify the

* Manuscript received December 7, 1965.

† Division of Protein Chemistry, CSIRO Wool Research Laboratories, Parkville, Vic.

[1] Canfield, R. E., and Anfinsen, C. B., "The Proteins." 2nd Edn, Vol. II. (Ed. H. Neurath.) (Academic Press: New York 1964.)

thiol groups of reduced proteins, although reagent concentrations or reaction periods 10–100 times greater than those usual with iodoacetamide would be required. The 4-bromo- and 4-iodobutanesulphonates are nearly equivalent alternatives. Using 0·1M 4-halobutanesulphonates in 0·4M Tris buffer at pH 8·6 and 20°, the 4-bromo compound reacted with *p*-nitrothiophenol at 90% and the 4-chloro at 7% of the rate observed with the 4-iodo compound. The immediate precursor in the synthesis of sodium 4-iodobutanesulphonate, 1,4-butanesultone, is in theory a more potent alkylating agent than the latter with the same end-products, and its use would avoid one synthetic step. However, a saturated solution of the sultone—6·5 g/100 ml (*c.* 0·5M) at 20°—reacted with thiol at a negligible rate at pH 8·6 and room temperature.

TABLE 1

SECOND-ORDER RATE CONSTANTS FOR THE REACTION BETWEEN *p*-NITROTHIOPHENOL AND IODO COMPOUNDS IN 0·4M TRIETHYLAMINE-HCl BUFFERS AT 20°

pH	Reactant	K (l. mole^{-1} sec^{-1})
9·2	iodoacetamide	$1{\cdot}5\times10^{-1}$
	4-iodobutanesulphonate	$6{\cdot}3\times10^{-3}$
	2-iodoethanesulphonate	$5{\cdot}1\times10^{-4}$
9·8	iodoacetamide	$1{\cdot}5\times10^{-1}$
	4-iodobutanesulphonate	$5{\cdot}3\times10^{-3}$
	2-iodoethanesulphonate	$3{\cdot}2\times10^{-4}$
10·4	iodoacetamide	$2{\cdot}4\times10^{-1}$
	4-iodobutanesulphonate	$4{\cdot}4\times10^{-3}$
	2-iodoethanesulphonate	$3{\cdot}1\times10^{-4}$
	iodomethanesulphonate	$<3{\cdot}8\times10^{-6}$*
11·0	iodoacetamide	$2{\cdot}9\times10^{-1}$
	4-iodobutanesulphonate	$4{\cdot}6\times10^{-3}$

* This is also the numerical value of the apparent pseudo-first-order constant for the disappearance of *p*-nitrothiophenol in the presence of 1M iodomethanesulphonate. Since the value of this constant for the spontaneous (? oxidative) disappearance of *p*-nitrothiophenol in the system was *c.* 4×10^{-6} sec^{-1}, the true value of the second-order constant is thus statistically indistinguishable from zero. The other values are all corrected for the spontaneous reaction.

Human serum albumin was treated at pH 8·6 according to the directions of Raftery and Cole,[2] except that the twentyfold excess of bromoethylamine hydrobromide of these authors was replaced by equivalent amounts of iodoacetamide or sodium 4-iodobutanesulphonate monohydrate. At 20°, the nitroprusside reaction was negative for iodoacetamide after a few mintues, for the sulphonate after 4–6 hr at pH 8·6. Both reactions were however continued for the same period (6 hr). The modified proteins were recovered by dialysis and lyophilization and hydrolysed with 6N HCl in a sealed evacuated tube. Comparison with a hydrolysate of the original

[2] Raftery, M. A., and Cole, D. R., *Biochem. Biophys. Res. Commun.*, 1963, **10**, 467.

protein showed that reduction of the cystine residues with mercaptoethanol had been incomplete, some 30% of the cystine remaining in both cases, the rest being replaced by *S*-carboxymethylcysteine or *S*-(4-sulphobutyl)cysteine. The latter was identified by comparing its chromatographic properties with those of a synthetic specimen (Fig. 2). The prolonged reaction period with excess iodoacetamide led to 85% loss of methionine and 50% loss of *S*-carboxymethylcysteine and the appearance of unidentified transformation products. No such effects were observable analytically for the sulphonate, although trace amounts of unknown substances were visible on the chromatogram.

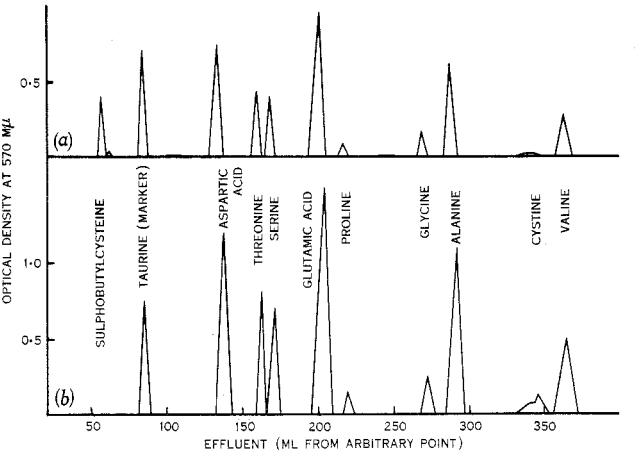

Fig. 2.—Transcript from Spinco Amino Acid Analyser of a portion of the elution curve of hydrolysates of reduced and sulphobutylated human serum albumin (*a*) and of the unmodified protein (*b*). (By the courtesy of Mr A. S. Inglis.)

Sulphobutylation is thus a feasible process for the preparation of modified proteins; the physical properties of sulphobutylated human serum albumin were altered from those of the original and the other modified forms of the reduced protein at least to the extent that it was no longer soluble on dialysis against distilled water.

Experimental

Sodium 4-Iodobutanesulphonate

Preparation of this compound has been reported[3] but with inadequate details and characterization. Sodium iodide (15·0 g, 0·1 mole) and 1,4-butanesultone (13·6 g, 0·1 mole) were refluxed in absolute ethanol (200 ml) for 1 hr. The ethanol was then removed under reduced pressure, and the residue recrystallized from ethanol/water (3 : 1; 50 ml) as white needles (25·5 g, 91%) in a micaceous felt, m.p. 266°, of sodium 4-iodobutanesulphonate monohydrate (Found: C, 16·0; H, 3·6; I, 41·2; S, 10·4. Calc. for $C_4H_8INaO_3S,H_2O$: C, 15·8; H, 3·3; I, 41·7; S, 10·5%). The product slowly discolours on exposure to light.

Sodium Salt of S-(*4-Sulphobutyl*)-L-*cysteine*

Sodium 4-iodobutanesulphonate (304 mg, 0·001 mole) and L-cysteine hydrochloride (157 mg, 0·001 mole) were dissolved in water (1 ml), triethylamine (0·5 ml) added, and the stoppered tube left at room temperature (24 hr). The mixture was reduced to dryness *in vacuo* and the residue extracted with boiling absolute ethanol (3 × 5 ml). The final residue was several times recrystallized from ethanol/water (3 : 1) to give the sodium salt of S-(*4-sulphobutyl*)-L-*cysteine* as white crystals, m.p. 252°, $[\alpha]_D^{29}$ −0·8° (*c*, 10; H_2O) (Found: C, 30·0; H, 5·4; N, 4·9; S, 22·7. $C_7H_{14}NNaO_5S_2$ requires C, 30·1; H, 5·1; N, 5·0; S, 23·0%).

[3] Jones, J. E., and Spence, J., U.S. Pat. 2,912,329 (1959).