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THIOLSULPHONATE DERIVATIVES OF AMINO ACIDS

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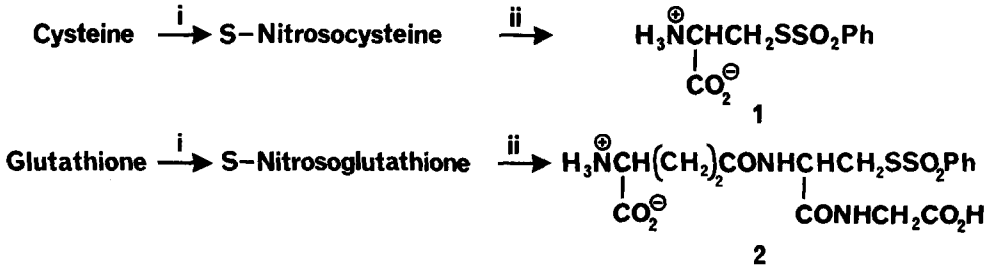
Summary : The synthesis and chemical properties of a series of amino acid derivatives containing the thiolsulphonate functionality are described.

In nature a wide variety of enzyme systems are dependent upon a nucleophilic sulphydryl functional group for their biological activity.[1] Hence compounds that can be targeted onto specific sulphydryl centres in vivo, and therefore act as selective enzyme inhibitors, may represent a viable approach to the design of useful therapeutic agents.

In a recent publication[2] the preparations of the S-phenylsulphonyl derivatives 1 and 2, of L-cysteine and glutathione respectively, were described (Scheme 1). Since the thiolsulphonate moiety is known[3] to be readily susceptible to nucleophilic attack, we hoped that by attaching it to a membrane permeable vehicle, such as an endogenous amino acid, we would have access to a series of chiral sulphenylating agents that would also possess valuable biological properties.



Scheme 1

i. $NaNO_2$,HCl ii. $PhSO_2Na$,HCl

We describe herein some of our results concerning the preparation and properties of the thiolsulphonate derivatives of cysteine and glutathione. In addition we felt it incumbent upon us to justify the original structure assignments[2] of S-phenylsulphonyl-L-cysteine 1 and S-phenylsulphonylglutathione 2. This was thought to be important because facile intramolecular 1,4 sulphur to nitrogen shifts of S-acylmercaptoethylamine derivatives have in the past presented well known ambiguities in the literature.[1,4]

In order to generate sufficient spectroscopic data for an unequivocal analysis, and also to demonstrate the general synthetic utility of the procedure, a series of analogues were prepared as shown in Table 1.[7]

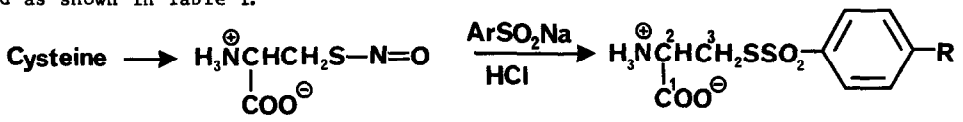
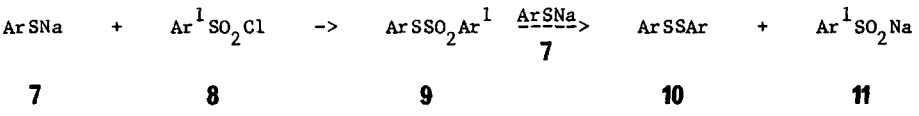


Table 1

Compound	R	^{13}C data			m.p. °C	Yield
		C-1	C-2	C-3		
1	H	170.92	54.27	36.54	155-158	59%
3	CH_3	170.96	54.37	36.57	150-151	33%
4	OCH_3	170.93	54.47	36.57	136-138	45%
5	CH_2CO_2H	171.15	54.55	36.79	140-142	60%
6	$NHCOCH_3$	171.25	54.59	35.80	174-176	42%

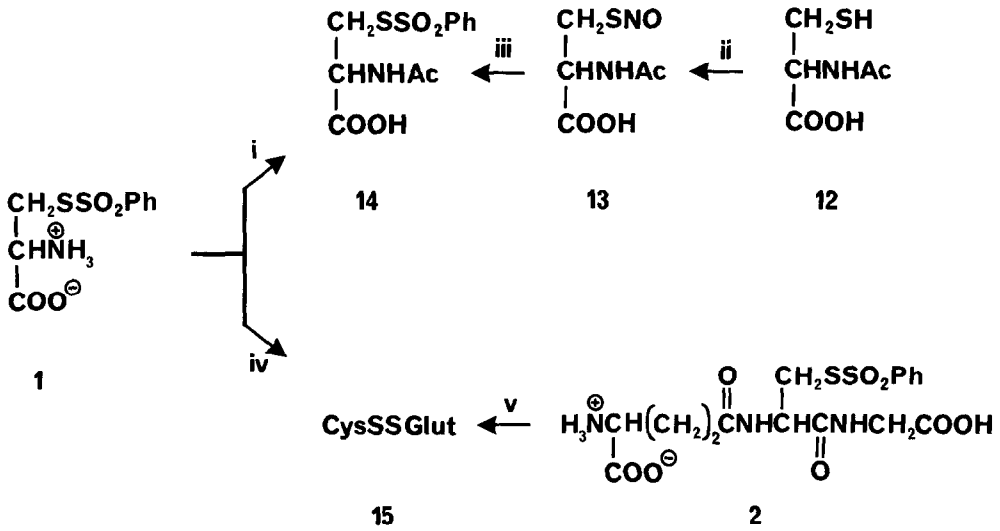
The thiolsulphonate analogues shown in Table 1 were synthesised using methodology similar to that previously described.[2] Though the ^{1}H n.m.r. spectra were compatible with the assigned structures, the most convincing spectroscopic evidence came from the ^{13}C n.m.r. data, where the ß-methylene of all the cysteinyl residues consistently showed a downfield shift of around 10.6-10.8 ppm. relative to that of cysteine, so signifying the presence of an S-substituted electron-withdrawing group, and in accord with attachment of a thiolsulphonate moiety.[5] The desired sodium salts of the sulphinic acids of type 11 were prepared in good yield by a slight modification of known literature procedures[6] as shown in scheme 2. Interestingly this method also demonstrates succinctly the difficulty in synthesing unsymmetrical thiolsulphonates of type 9, since the latter, formed as reactive intermediates during this reaction, undergo a far more rapid reaction with the original thiol reagent 7 than does the suphonyl chloride 8.



Scheme 2

Thus, as a representative example, the reaction of two equivalents of the sodium salt of p-toluenethiol with p-toluenesulphonyl chloride in ethanol at 50°C for 50 minutes gave after cooling, filtration, evaporation and subsequent treatment with water, the water insoluble, symmetrical 4,4'-dimethyldiphenyldisulphide, which was discarded. Concentration of the aqueous filtrate in vacuo, however, afforded the desired sodium salt of p-toluenesulphinic acid in 61% yield, contaminated with a small amount of sodium chloride.

In an alternative, chemically derived structure analysis,[7] it was first shown that N-acetyl-S-phenylsulphonyl-L-cysteine 14 (m.p. 165-167°C, from MeOH-Ether) could be prepared by two separate routes, as shown in scheme 3. Thus conversion of N-acetyl-L-cysteine 12 to the corresponding S-nitroso derivative 13, followed by trapping *in situ* with benzenesulphinic acid afforded 14 in 54% overall yield. An identical product (mixed melting point, ^{1}H and ^{13}C n.m.r.) was also obtained by careful acetylation of 1 (acetic anhydride, tetrahydrofuran, reflux 2 hours, 67%).



Scheme 3

i. Ac_2O, THF, reflux ii. HNO_2 iii. $PhSO_2H$ iv. Glutathione, $NaHCO_3$ v. Cysteine, $NaHCO_3$

From these results it seems difficult to avoid concluding, without invoking multiple S→N and N→S rearrangements, that the S-phenylsulphonyl-L-cysteine assignment, originally proposed for 1, must be correct.

Since it has already been demonstrated that thiolsulphonates undergo a facile reaction with thiols to form unsymmetrical disulphides, it was anticipated that 1 and 2 would both be susceptible to similar nucleophilic displacements. Hence reaction of 1 with stoichiometric quantities of glutathione and sodium bicarbonate in water afforded the biologically important[8,9] mixed disulphide, cysteinyl-glutathione[10] 15 in 86% yield.

In complementary fashion the reaction of S-phenylsulphonylglutathione 2 with L-cysteine under similar conditions formed the same disulphide 15 in 75% yield. The optical rotation of 15 corresponds, within experimental error, to published values,[9] and hence we surmise that the preparations and subsequent reactions of the thiolsulphonates 1 and 2 proceed without loss of chirality.

Thus we conclude that the compounds designated 1 and 2, on the available evidence, are most likely to be the S-phenylsulphonyl derivatives of L-cysteine and glutathione respectively, and that derivatives of this type should find widespread application as valuable reagents for the preparation of mixed disulphides both *in vivo* and *in vitro*.

EXPERIMENTAL

Preparation of S,S'-L-cysteinylglutathione

To a stirred mixture of glutathione (0.307 g, 1.0 mmol) and S-phenylsulphonyl-L-cysteine (0.261 g, 1.0 mmol) in argon-degassed water (10 cm^3) was added sodium bicarbonate (0.084 g, 1.0 mmol) at 20°C. The initial suspension quickly formed a solution, which after 1 hour was cooled to 5°C and maintained at this temperature for a further 20 hours. The resulting fine white solid was filtered off then washed successively with water (3 x 1 cm^3), acetone (3 x 4 cm^3) and ether (2 x 10 cm^3) to afford cysteinyl-glutathione[10] (0.32 g, 0.86 mmol, 86%) m.pt. 234°C (decomp.) (lit, 234°C); $[\alpha]_D^{25}$ -110 (c, 0.25 in 1.0 M HCl); δ_C (50.31 MHz; D_2O-DCl (pD 1); standard, DSS) 27.90, 33.55, 39.42, 40.87, 43.76, 54.26, 54.70, 55.00, 172.81, 173.78, 174.94, 175.34, 176.94. (Found : C, 36.6; H, 5.19; N, 13.1. Calc for $C_{13}H_{22}N_4O_8S_2$: C, 36.6; H,5.2; N,13.1%)

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7. Satisfactory spectroscopic and analytical data were obtained on all new compounds. Yields were not optimised. All ^{13}C spectra were run on a Varian XL-200 M.Hz N.M.R. spectrometer using D_2O-DCl (pD 1) as solvent at 25% w/v.
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