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SOME OBSERVATIONS CONCERNING THE S-NITROSO AND S-PHENYLSULPHONYL DERIVATIVES OF L-CYSTEINE AND GLUTATHIONE

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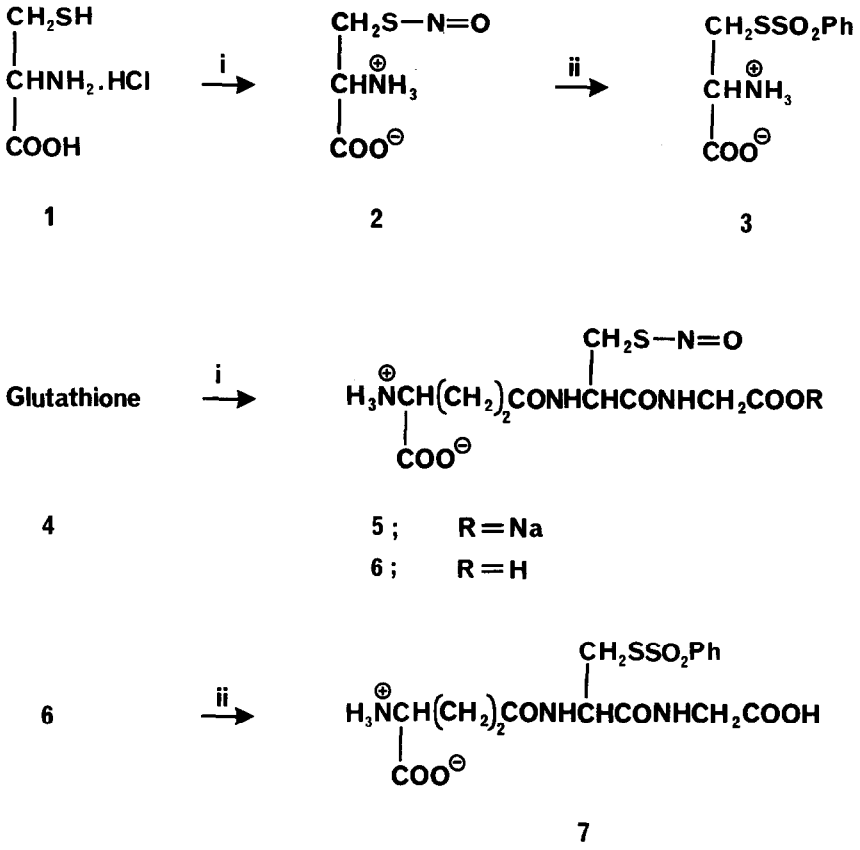
Summary : The preparation of the thiolsulphonate derivatives 3 and 7 of L-cysteine and glutathione, respectively, via their corresponding S-nitroso derivatives 2 and 6, is described.

Over the last few years close attention has been paid to the potent chemical and biological properties of compounds possessing the S-nitroso[1] and thiolsulphonate[2] functional groups. The former are, in general, powerful nitrosating species,[3] while both moieties are also synthetically useful sulphenylating agents,[4] and hence potentially valuable biological tools. Herein described is a one-pot synthesis of the thiolsulphonate derivatives 3 and 7 from the ubiquitous amino acids, L-cysteine[1], and glutathione 4, respectively. The procedure utilises the synthetic potential of the corresponding highly reactive S-nitroso derivates 2 and 6, both of which have recently been reported as displaying modulatory roles in the control of enzyme activity[5] and are also heavily implicated in the formation of N-nitrosamines in vivo.[6]

Thus treatment of L-cysteine hydrochloride hydrate with sodium nitrite and hydrochloric acid afforded the unstable S-nitroso derivative 2 in situ, as previously described.[7] However, when the crude acidic reaction mixture was treated with sodium benzenesulphinate the corresponding thiolsulphonate derivative 3 was formed in 42% yield, without recourse to the isolation of 2. The corresponding thiolsulphonate derivative 7 of glutathione 4 was prepared in similar fashion in 40% yield, although, somewhat surprisingly, it was found that the presence of mineral acid was not essential for the initial generation of nitrous acid, since the sodium salt of S-nitrosoglutathione 5 is apparently readily formed. All attempts to isolate and characterise the unstable, highly hygroscopic, sodium salt 5 failed.

Presumably the S-nitroso derivative 5 is formed as a consequence of glutathione being not only acidic enough to protonate nitrite ion, but also sufficiently nucleophilic to intercept the nitrosating species so formed. The 'suicide nature' of this reaction was confirmed when it was found that, in the presence of excess reduced glutathione, substantial amounts of disulphide formation were observed. Since it is well known that S-nitroso compounds readily react with thiols to form the corresponding disulphides,[13a] it is interesting to speculate that, these in vitro observations, in conjunction with previously reported results,[8] may represent another example of the established cytoprotective role that glutathione plays in nature.



Scheme 1

i) $NaNO_2$ ii) $PhSO_2H$

In direct contrast to S-nitrosocysteine, which is both difficult to purify and also to characterise, [6,9] S-nitrosoglutathione 6 could be readily isolated, but only as a partial hydrate.

In parallel with literature reports, [6, 10, 11] it was subsequently shown that glutathnione preferentially reacts with nitrous acid in the presence of N-methylaniline at various acidic pH levels. However, any potential role for glutathione as a possible inhibitor of N-nitrosamine formation in vivo must inevitably be tempered by the ability of 6 to transnitrosate in non-acidic media. [6, 12] Interestingly the initial incorporation of excess ascorbic acid in the reaction medium largely prevented S-nitrosoglutathione formation. Hence it seems most probable that dietary ascorbic acid, a known scavenger of nitrite ion, [12, 13]

is in the first line of defence against N-nitrosamine formation in vivo, while glutathione 4, must play a fundamentally more complex, double-edged role [11, 13].

Experimental[14]

Preparation S-phenylsulphonyl-L-cysteine, 3.

To a stirred ice-cold solution of L-cysteine hydrochloride hydrate (3.5 g., 20 mmol) in 2N HCl (20 cm^3) was slowly added a solution of sodium nitrite (1.38 g., 20 mmol) in water (10 cm^3). After 40 minutes at 5°C the deep red solution was treated with a solution of sodium benzenesulphinate (6.5 g., 40 mmol) in water (40 cm^3) and stirred for a further 90 minutes. The resulting precipitate was filtered off tnen washed successively with water (2 x 40 cm), acetone (2 x 30 cm) and ether (2 x 30 cm). Recrystallisation from water afforded pure S-phenylsulphonyl-L-cysteine, 3 (3.1 g., 11.9 mmol, 59%) m.p. 155-158°C (decomp.), (Found: C, 41.3; H, 4.4; N, 5.3; S, 24.9; $C_9 H_{11} NO_4 S_2$ requires C, 41.4; H, 4.2; N, 5.4; S, 24.5%).

Preparation of S-phenylsulphonylglutathione, 7.

To a stirred ice-cold solution of glutathione (2.5 g., 8.1 mmol) in water (20 cm^3) was added a solution of sodium nitrite (0.55 g., 8.1 mmol) in water (10 cm^3). After 15 minutes the deep red solution was treated first with a solution of sodium benzenesulphinate (2.6 g., 16 mmol) in water (12 cm^3) and then with 2N HCl (8 cm^3). The resulting mixture was stirred for 20 hours at 20°C after which time the precipitated solid was filtered off and washed successively with water (2 x 10 cm^3), acetone (2 x 20 cm^3) and ether (2 x 20 cm^3). Recrystallisation from water afforded pure S-phenylsulphonylglutathione dinydrate, 7 (1.6 g., 3.3 mmol, 41%) m.p. 176-179°C (decomp.), (Found C, 39.8; H, 5.27; N, 8.7; S, 13.5; H_2O, 7.5. $C_{16} H_{21} N_3 O_8 S_2 \, 2H_2O$ requires C, 39.75; H, 5.28; N, 8.7; S, 13.3; H_2O 7.4%).

Preparation of S-nitrosoglutathione, 6.

To a stirred ice-cold solution of glutathione (1.53 g., 5 mmol) in water (8 cm^3) containing 2N HCl (2.5 cm^3) was added in one portion sodium nitrite (0.345 g., 5 mmol). After 40 minutes at 5°C the red solution was treated with acetone (10 cm^3) and stirred for a further 10 minutes. The resulting fine pale red precipitate was filtered off and then washed successively with ice-cold water (5 x 1 cm^3), acetone (3 x 10 cm^3) and ether (3 x 10 cm^3) to afford S-nitrosoglutathione (1.29 g., 3.8 mmol, 76%) (λ max.) (H_2O) 335, 545 nm (ϵ 922, 15.9 $dm^3\ mol^{-1}\ cm^{-1}$); $[\alpha]_D^{24}$ + 47 (c 1.31 in H_2O); Found: C, 35.1; H, 4.8; N, 16.4; S, 9.4; H_2O 1.4. $C_{10} H_{16} N_4 O_7 S$ 0.25 H_2O requires C, 35.2; H, 4.9; N, 16.4; S, 9.4; S, 9.4; H_2O, 1.3%).

REFERENCES

1. For a recent review see S.Oae and K. Shinhama, Org.Prep.Proc.Int., 1983, 15(3), 165.
2. L.A. Field and R. Ravindranathan, J.Org.Chem., 1979, 44, 2624 and refs therein.
3. S.Oae, D.Fukushima and Y.H.Kim, J.Chem.Soc.Chem.Commun., 1977, 406.
4. (a) G. Palumbo and R. Caputo, Synthesis, 1981, 888.
   (b) B.M. Trost, Chem.Rev., 1978, 78, 363.
   (c) S. Oae, Y.H. Kim, D. Fukushima and K. Shinhama, J.Chem.Soc. Perkin Trans. 1, 1978, 913.
5. L.J. Ignarro, B.K. Barry, D.Y. Gruetter, J.C. Edwards, E.H. Ohlstein, C.A. Gruetter and W.H. Baricos, Biochem.Biophys.Res.Commun., 1980, 94, 93.
6. (a) R.C. Massey, M.J. Dennis, C. Crews, D.J. McWeeny and R. Davies, Int. Agency Res. Cancer Sci.Publ., 1980, 31, 291 and refs therein.
   (b) R. Davies, M.J. Dennis, R.C. Massey and D.J. McWeeny, Int. Agency Res. Cancer Sci.Publ., 1978, 19, 183.
   (c) M.J. Dennis, R. Davies and D.J. McWeeny, J.Sci.Food Agric., 1979, 30, 639.
7. (a) B. Saville, Analyst, 1958, 83, 670.
   (b) A. Mirna and K. Hofmann, Fleischwirtschaft, 1969, 49, 1361.
   (c) C. Cantoni, M.A. Bianchi, G. Beretta, Ind. Aliment. (Pinerolo, Italy), 1975, 14(7-8), 79.
8. (a) L.F. Chasseud, Adv.Cancer Res., 1979, 29, 1975.
   (b) W.G. Stillwell, Trends Pharm. Sci., 1981, 250.
   (c) E.M. Kosower and N.M. Kosower, Nature, 1969, 224.
9. U. Schultz and D.R. McCalla, Can.J.Chem., 1969, 47, 2021.
10. N.P. Sen and B. Donaldson, Int. Agency Res. Cancer Sci. Publ., 1975, 9, 103.
11. S.C. Boyd, H.A. Sasame and M.R. Boyd, Science, 1979, 20, 1010.
12. B.C. Challis and J.A. Challis; in 'The Chemistry of Functional Groups', Supplement F : the chemistry of amino, nitroso and nitro compounds and their derivatives. ed. S. Patai, 1982, pp 1151-1223. John Wiley and Sons. New York.
13. (a) S.S. Mirvish, L. Wallcave, M. Eagen and P. Shubik, Science, 1977, 177, 65.
   (b) C. Lintas, A. Clark, J. Fox, S.R. Tannenbaum and P.M. Newberne, Carcinogenesis (London), 1982, 3(2), 161 and refs therein.
14. Full spectroscopic analyses will be published in a following publication.

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