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MIXED DISULFIDES OF L-CYSTEINE AND ITS DERIVATIVES WITH 2-MERCAPTOETHANOL

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- Skarzewski, *Synthesis*, 401 (1994) and references cited therein.
- a) J. M. Khurana, P. K. Sahoo, S. S. Titus and G. C. Maikap, *Synth. Commun.*, **20**, 1357 (1990), and references cited therein; b) J. M. Khurana, A. Ray and P. K. Sahoo, *Bull. Chem. Soc. Jpn.*, **67**, 1901 (1994).
 - S. Buckingham, (Ed.) "*Dictionary of Organic Chemistry*", 5th edition, Chapman and Hall, New York, 1988.
 - US Patent, 2682544 (1954); *C.A.*, **49**, P7006a (1955)
 - M. Uchino, K. Suzuki and M. Sekiya, *Chem. Pharm. Bull. Jpn.*, **26**, 1837 (1978).
 - A. I. Vogel "*Quantitative Inorganic Analysis*", p. 396, ELBS/Longman, 1975.
 - A. I. Vogel's "*Textbook of Practical Organic Chemistry*", 4th ed., p. 374, ELBS/Longman, 1987.
 - J. M., Khurana and P. K. Sahoo, *Synth. Commun.*, **22**, 1691 (1992).
 - Ref. 9, p. 587.
 - "*Methoden der Organische Chemie*", Houben Weyl, **9**, 136 (1955).

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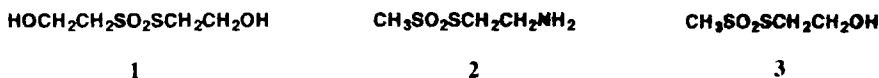
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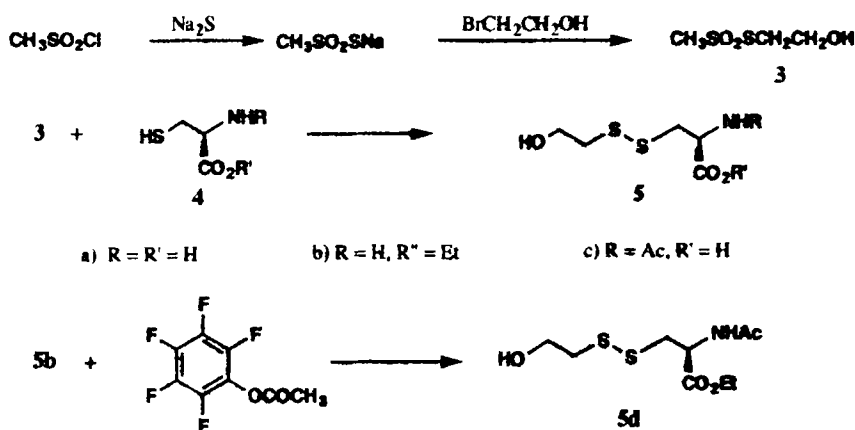
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The mixed disulfide (**5a**), of L-cysteine (**4a**) and 2-mercaptoethanol (ME) has been shown to function as a transport form of L-cysteine in mouse lymphoma cells in culture.¹ Whereas L-cysteine was not taken up by these cells, the presence of ME in the medium resulted in a thiol-disulfide exchange reaction producing **5a**. This compound was taken up by the lymphoma cells via the "L" amino acid transport system and the disulfide bond subsequently reduced by intracellular enzymes to liberate L-cysteine, thereby promoting cell growth. Murray and Rathbun² have also observed that not



only was the uptake of L-cysteine (from L-cystine) increased in rat ocular lenses *in vitro*, glutathione biosynthesis was also stimulated by the addition of ME to the medium and implicated the mixed disulfide **5a** as the transport form of L-cysteine in lens. Thus, **5a** can be considered to be a pro-drug of L-cysteine, and, like other cysteine pro-drugs,³ may exhibit protective effects against toxic xenobiotics that are metabolized to reactive intermediates. Accordingly, large quantities of **5a** as well as its radioactive form with a long half life were desired for *in vivo* metabolic studies.

The published method¹ for the preparation of the regioisomeric [³⁵S]-labeled **5a** by thiol-disulfide exchange reactions is not efficient for our projected radioactive synthesis of the molecule using L-[¹⁴C]cysteine which must be prepared from the commercially available L-[¹⁴C]cystine. Other oxidative methods that utilize mixtures of the free thiols and air (Fe³⁺ catalyzed)⁴ or cyanogen bromide⁵ are equally not feasible. Moreover, a general method applicable for the preparation of the mixed disulfide forms of ME with carboxyl- and amino-protected cysteine derivatives (**5b, c, d**, Scheme 1) was desired. Two compounds described in the literature wherein ME is functionalized as thiol-sulfonates, *viz.*, S-(2-hydroxyethyl)-methanethiol-sulfonate (**3**)⁶ and S-(2-hydroxyethyl)-2-hydroxyethanethiol-sulfonate (**1**)⁷, appeared to be ideal as general synthons for the projected syntheses of **5**. These synthons are similar to S-(2-aminoethyl)-methanethiol-sulfonate (**2**)⁸ which has been used extensively to prepare the mixed disulfides of 2-mercaptoethylamine with protein sulfhydryl groups,



Scheme 1

or with the sulfhydryl groups of biological molecules such as glutathione or cysteine derivatives. Of these, compound **3** appeared to be better characterized, and **3** was selected for use as a general synthon for the preparation of compounds **5a, b, and c** according to Scheme 1.

Since the purification of the intermediate sodium methanethiol-sulfonate (Scheme 1) was cumbersome,^{6,8} the crude product was not purified but was used directly in the next step to prepare

pure **3** without compromising its overall yield, and **3** was obtained, after distillation, in a respectable 23% yield from sodium sulfide. Reaction of L-cysteine with the synthon **3** in aqueous solution at pH 6.3, gave **5a** in 41% yield after recrystallization (Scheme 1). Under the same conditions, but using L-cysteine ethyl ester (**4b**) and/or *N*-acetyl-L-cysteine (**4c**) and slightly different work-up conditions, the corresponding mixed disulfides **5b** and **5c** were prepared. For the preparation of **5d**, it was more convenient to chemoselectively acetylate **5b** with pentafluorophenyl acetate⁹ than to follow Scheme 1 with *N*-acetyl-L-cysteine ethyl ester.

All of the target compounds (except for the known **5a**) were characterized by NMR spectra, mass spectra, optical rotations and by elemental analyses. They will be evaluated as hepatoprotective³ and anticataract¹⁰ agents in rodents. The synthesis of ¹⁴C-labeled **5a** will be patterned after the present procedure.

EXPERIMENTAL SECTION

¹H NMR spectra were recorded at ambient temperature on either GE-300 or Bruker AC-200 NMR spectrometers. Chemical shifts are reported as δ values (ppm). Mass spectra (CI, positive ion) were obtained on a Kratos MS 25 mass spectrometer. For TLC analyses, Analtech silica gel GF plates were used and the solvent system was butanol:acetic acid:water (50:11:25) or propanol:water (90:10 to 70:30). The plates were visualized by spraying with ninhydrin or ceric sulfate solution and heating. Column chromatography was carried out using columns packed with Kieselgel 60 (230-400 mesh) silica gel (EM Science). Pentafluorophenyl acetate was purchased from Sigma Chemical Co. Whenever the reactants or products contained a free sulfhydryl group, the reactions were conducted under a N₂ atmosphere.

S-(2-Hydroxyethylmercapto)-L-cysteine (5a). (2-Mercaptoethanol/L-Cysteine Mixed Disulfide).

A stirred solution of L-cysteine (1.57 g, 13.0 mmol) in H₂O (70 mL) was adjusted to pH 6.3 with 2N aq. NaOH. To this was added a solution of synthon **3** (2.54 g, 16.3 mmol)^{6,8} in 25 mL H₂O over 10 min. The reaction mixture was stirred overnight and then extracted with 3 x 50 mL portions of ethyl acetate to remove excess synthon and other by-products. The pH was then adjusted to 5.4 with 2N aq. NaOH and the solvent was removed using a rotary evaporator. The resulting semi-solid was taken up in 50 mL hot H₂O and the cloudy solution was clarified by filtration. About 100 mL hot ethanol was added to the filtrate until precipitation began. The mixture was allowed to cool to room temperature and kept in a refrigerator at 5°. The product that precipitated was collected and rinsed with 30% aq. ethanol and dried to give 1.04 g (41% yield) of a white solid, mp. 179-181°, lit.⁷ 161-162°, lit.⁵ 188-189° after drying in a desiccator over P₂O₅; $[\alpha]_D^{24} = -164^\circ$ ($c = 0.6$ in 1 N aq. HCl) (lit.^{5,7} = 137°-141°). A sample of **5a** prepared using the cyanogen bromide method⁵ had a mp of 176.5-179° and $[\alpha]_D^{23} = -157^\circ$ ($c = 0.8$ in 1 N aq. HCl). A second crop of less pure material (0.13 g, 5%) was also recovered.

Ethyl S-(2-Hydroxyethylmercapto)-L-cysteinate (5b). (2-Mercaptoethanol/L-Cysteine Ethyl Ester Mixed Disulfide).

Cysteine ethyl ester hydrochloride (1.94 g, 10.4 mmol) in H₂O (60 mL) and synthon **3** 2.05 g, 13.1 mmol) in 25 mL H₂O were reacted as above for **5a**. The pH which was 2.0 after overnight reaction was adjusted to 1.5 with 1 N aq. HCl and the solution was saturated with solid

NaCl before extraction with 3 x 75 mL of ethyl acetate. The combined ethyl acetate extracts were dried (Na_2SO_4) and the solvent evaporated to give 0.46 g of oil which was mostly unreacted **3** and mercaptoethanol disulfide by TLC and NMR analysis. The aqueous phase was basified to pH 7.5 with 2N aq. NaOH, resaturated with solid NaCl and extracted with 3 x 100 mL portions of CHCl_3 . The combined CHCl_3 extracts were dried (Na_2SO_4) and the solvent removed to give, after drying in vacuo, 1.68 g of the titled compound as a colorless oil (72% yield). ^1H NMR (CDCl_3): δ 1.27 (t, 3H, CH_3CH_2), 2.31 (br s, 3H, NH_2 and OH- exchanges with D_2O) 2.86 (t, J = 5.7 Hz, 2H, $\text{CH}_2\text{CH}_2\text{OH}$), 2.91 (dd, J = 7.4 Hz, J = 13.7 Hz, 1H, CHCHCO_2), 3.11 (dd, J = 4.5 Hz, J = 13.7 Hz, 1H, CHCHCO_2), 3.77 (dd, J = 4.5 Hz, J = 7.4 Hz, 1H, CHNH_2), 3.85 (t, J = 5.8 Hz, 2H, CH_2OH) 4.18 (q, J = 7.1 Hz, 2H, CH_2CH_3). Mass spectrum (CI) m/z 226 (MH^+) $[\alpha]_{\text{D}}^{24} = +7.4$ (c = 1.1, MeOH).

Anal. Calcd. for $\text{C}_7\text{H}_{15}\text{NO}_3\text{S}_2$: C, 37.31; H, 6.71; N, 6.22; S, 28.46.

Found: C, 37.15; H, 6.50; N, 6.00; S, 28.76.

Ethyl S-(2-Hydroxyethylmercapto)-N-acetyl-L-cysteinate (5d). To a stirred solution of ethyl S-(2-hydroxyethylmercapto)-L-cysteinate (**5b**) (830 mg, 3.69 mmol) in dry DMF (10 mL) was added a solution of pentafluorophenyl acetate⁹ (1.11 g, 4.90 mmol) in 5 mL dry DMF. The solution was stirred overnight when TLC analysis showed the absence of **5b**. The solvent was then evaporated on a rotary evaporator and solvent evaporation was repeated several times after the addition of ethanol and toluene each time to remove DMF. The crude product was purified by column chromatography on silica gel. Elution with ethyl acetate gave 0.87 g of colorless oil (88.3% yield). ^1H NMR (CDCl_3): δ 1.29 (t, 3H, CH_3CH_2), 2.05 (s, 3H, CH_3CO), 2.6 (s, 1H, OH- exchanges with D_2O) 2.88 (t, J = 5.6 Hz, 2H, $\text{CH}_2\text{CH}_2\text{OH}$), 3.18 (dd, J = 5.1 Hz, J = 14.2 Hz, 1H, CHCHCO_2), 3.24 (dd, J = 5.2 Hz, J = 14.2 Hz, 1H, CHCHCO_2), 3.86 (t, J = 5.7 Hz, 2H, CH_2OH) 4.23 (q, J = 7.2 Hz, 2H, CH_2CH_3), 4.88 (td, J = 5.1 Hz, J = 7.4 Hz, 1H, CHNH_2), 6.42 (br d, 1H, NH); mass spectrum, (CI) m/z 268 (MH^+). $[\alpha]_{\text{D}}^{24} -77.6^\circ$ (c = 1.0, MeOH).

Anal. Calcd. for $\text{C}_9\text{H}_{17}\text{NO}_4\text{S}_2$: C, 40.43; H, 6.41; N, 5.24; S, 23.98.

Found: C, 40.39; H, 6.49; N, 5.22; S, 23.76.

N-Acetyl-S-(2-Hydroxyethylmercapto)-L-cysteine (5c). N-Acetylcysteine (0.89 g, 5.45 mmol) in H_2O (30 mL) and synthon **3** (1.10 g, 7.05 mmol) in 10 mL H_2O were reacted as above for **5a**. The pH was adjusted to 9 with 2N aq. NaOH and the mixture saturated with solid NaCl. After readjusting the pH to 9.1 the mixture was extracted with 3 x 40 mL of ethyl acetate to remove excess synthon. The aqueous phase was acidified to pH 1.9 with 1N aq. HCl, resaturated with solid NaCl and the mixture was extracted with 3 x 60 mL of ethyl acetate. The combined ethyl acetate extracts were dried (Na_2SO_4) and the solvent removed to give 1.05 g of thick oil. This was purified by column chromatography on silica gel eluting with propanol:1 N aq. HCl:ethyl acetate (5:1:94 to 25:1:74) to give 453 mg of **5c** as a thick, colorless oil (34.7% yield). ^1H NMR (CD_3COCD_3): δ 1.97 (s, 3H, CH_3CO), 4.1 (br s, 1H, OH-) 2.89 (t, J = 6.4 Hz, 2H, $\text{CH}_2\text{CH}_2\text{OH}$), 3.07 (dd, J = 8.2 Hz, J = 13.8 Hz, 1H, CHCHCO_2), 3.28 (dd, J = 4.9 Hz, J = 13.8 Hz, 1H, CHCHCO_2), 3.78 (t, J = 6.4 Hz, 2H, CH_2OH) 4.1 (br s, 1H, OH) 4.77 (m, 1H, CHNH_2), 7.6 (br d, 1H, NH). Mass spectrum (CI) m/z 240 (MH^+) $[\alpha]_{\text{D}}^{24} -87.2^\circ$ (c = 1.0, MeOH).

Anal. Calcd. for $C_7H_{13}NO_4S_2$: C, 35.13; H, 5.48; N, 5.85; S, 26.80.

Found: C, 34.89; H, 5.52; N, 5.68; S, 26.65.

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REFERENCES

1. T. Ishii, S. Bannai and Y. Sugita, *J. Biol. Chem.*, **256**, 12387 (1981).
2. D. L. Murray and W. B. Rathbun, *Curr. Eye Res.*, **9**, 55 (1990).
3. H. T. Nagasawa, D. J. W. Goon, W. P. Muldoon and R. T. Zera, *J. Med. Chem.*, **27**, 591 (1984).
4. J. P. Casey and R. B. Martin, *J. Am. Chem. Soc.*, **94**, 6141 (1972).
5. O. Abe, M. F. Lukacovic and C. Ressler, *J. Org. Chem.*, **39**, 253 (1974).
6. Z. Shaked, R. P. Szajewski and G. M. Whitesides, *Biochem.*, **19**, 4156 (1980).
7. A. Schoberl and H. Grafje, *Ann.*, **617**, 71 (1958).
8. T. W. Bruice and G. L. Kenyon, *J. Protein Chem.*, **1**, 47 (1982).
9. L. Kisfaludy, T. Mohacsi, M. Low and F. Drexler, *J. Org. Chem.*, **44**, 654 (1979).
10. W. B. Rathbun, A. M. Holleschau, J. F. Cohen and H. T. Nagasawa, *Invest. Ophthalm. Vis Sci.*, In press, **1996**.
