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Facile synthesis of (*R*)N-2-hydroxyacyl-L-cysteine derivatives: (*R*)N-2-hydroxyacyl transfer from enzymatically-synthesized (*R*)S-2-hydroxyacylglutathione derivatives to L-cysteine

Short Communication

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Summary. N-(*R*)-2-Hydroxyacyl-L-cysteine derivatives were conveniently synthesized by the reaction of the corresponding S-(*R*)-2-hydroxyacylglutathione with cysteine. The (*R*)2-hydroxyacyl group was transferred from the S-glutathionyl moiety to S-cysteinyl, forming the corresponding (*R*)S-2-hydroxyacylcysteine; this rearranged to the (*R*)N-hydroxyacylcysteine. These compounds have anti-proliferative activity associated with the inhibition of *de novo* pyrimidine synthesis.

Keywords: Amino acids – N-2-Hydroxyacylcysteine – S-2-Hydroxyacylglutathione – Glyoxalase – Glutathione – Cysteine

Abbreviations: TRIS, tris(hydroxymethyl) aminomethane; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

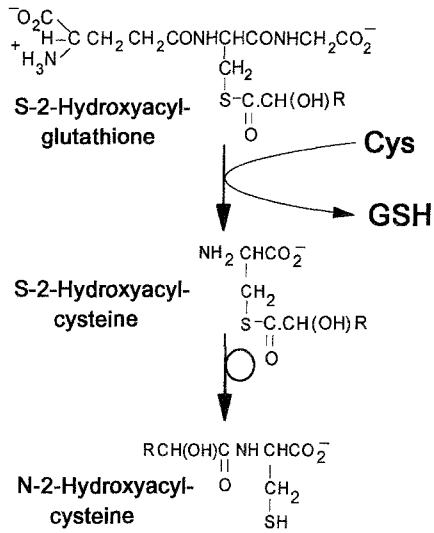
The synthesis of (*R*)N-2-hydroxyacyl-L-cysteine derivatives is normally a complex process involving preparation of the appropriate 2-oxoacyl chloride (Ottenheijm and de Man, 1975), reaction of the 2-oxoacyl chloride with L-cystine, reduction of N,N′-bis(2-oxoacyl)-L-cystine to N-(2-hydroxyacyl)-cysteine and separation of the *R*- and *S*- isomers. (*R*)N-2-Hydroxyacyl-L-cysteine derivatives are of interest as intermediates in the extracellular metabolism of S-2-hydroxyacylglutathione derivatives. N-D-Lactoylcysteine is formed from the metabolism of S-D-lactoylglutathione by γ-glutamyl transferase and dipeptidase, and has been implicated in the anti-leukaemia activity of S-D-lactoylglutathione (Edwards and Thornalley, 1994; Clelland et al., 1992). The anti-leukaemia activity is mediated by inhibition of *de novo* pyrimidine synthesis where the prospective mechanism of action is inhibition of the zinc metalloenzyme dihydro-orotase by N-D-lactoylcysteine (Edwards and Thornalley, 1994). Dihydro-orotase activity (EC 3.5.2.3) is associated

with part of the multi-enzyme protein CAD which catalyzes the first three steps of committed *de novo* pyrimidine synthesis (Evans et al., 1993). Dihydro-orotase catalyzes the formation of dihydro-orotate from N-carbamoylaspartate. It is susceptible to inhibition by N-acyl-L-cysteine derivatives which are presumed to proffer a potent thiol ligand to the prosthetic zinc ion and inhibit the enzyme (Christopherson et al., 1989).

A possible approach to the facile synthesis of N-2-hydroxyacylcysteine derivatives was by acyl transfer from the corresponding S-2-hydroxyacylglutathiones. S-2-Hydroxyacylglutathione derivatives were conveniently prepared by enzymatic synthesis from α-oxoaldehydes and reduced glutathione, catalyzed by glyoxalase I (EC 4.4.1.5) (Clelland and Thornalley, 1991). Previous studies (Tate, 1975) had shown that the *in situ* generation of N-unblocked S-acylcysteinyl derivaties was followed by rapid intramolecular acyl transfer to form the corresponding N-acylcysteinyl derivative. Also, the slow kinetics of the spontaneous hydrolysis of S-2-hydroxyacylglutathione derivatives at pH 7.4 (Reeves and Thornalley, 1993) suggested that it should be possible to achieve reaction conditions where the transfer of the acyl group from S-2-hydroxyacylglutathione to cysteine was favoured over hydrolysis of the thiolester.

(*R*)N-2-Hydroxyacylcysteine derivatives were conveniently synthesized in high yield by (*R*)-2-hydroxyacyl transfer from the corresponding (*R*)S-2-hydroxyacylglutathione to cysteine with rearrangement of the S-(*R*)-2-hydroxyacylcysteine formed *in situ* to the (*R*)N-hydroxyacylcysteine (Scheme 1). Although the proposed intermediate (*R*)S-2-hydroxyacylcysteine was not detected, its formation *in situ* is probable given the greater nucleophilicity of the thiol group than that of the α-amino group of cysteine, and the precedent of the formation of N-D-lactoylcysteinyl-glycine from S-D-lactoylglutathione when the γ-glutamyl residue blocking the cysteinyl α-amino group was removed by γ-glutamyl transferase (Tate, 1975). Indeed, an alternative procedure for the synthesis of (R)S-2-hydroxyacylcysteine derivatives was a 2-step enzymatic procedure with γ-glutamyl transferase-catalyzed conversion of (*R*)S-2-hydroxyacylglutathione to (*R*)N-2-hydroxyacylcysteinyl-glycine, followed by dipeptidase-catalyzed conversion of (*R*)N-2-hydroxyacyl cysteinylglycine to (*R*)S-2-hydroxyacylcysteine (Tate, 1975; Edwards and Thornalley, 1994). Having tried the first step with commercial γ-glutamyl transferase however, we obtained only poor yields (<10%) of the (*R*)N-2-hydroxyacylcysteinylglycine derivative; the majority of the product was reduced glutathione and the corresponding (R)-aldonic acid. This may indicate that commercial γ-glutamyl transferase has thiol esterase activity. Hence, the acyl transfer reaction to cysteine was preferred.

(*R*)N-2-Hydroxyacyl-L-cysteine derivatives are of interest as prospective inhibitors of dihydro-orotase and they have anti-proliferative activity associated with the inhibition of de novo pyrimidine synthesis (Edwards and Thornalley, 1994).



Scheme 1. Synthesis of (*R*)N-2-Hydroxyacyl-L-cysteine derivatives

Table 1. NMR Spectra of (*R*)N-2-hydroxyacylcysteine derivatives in D_2O

Compound	N-D-Lactoyl-cysteine	N-D-Mandelyl-cysteine	N-D-Glyceroyl-cysteine
Proton NMR spectra			
Assignment δ (J)			
Cysteinyl			
2-H	4.45	4.48	4.50
3A-H	2.88	2.88	2.88
3B-H	2.81	2.81	2.82
($J_{2,3A}$	2.9	4.7	5.1
($J_{2,3B}$	6.6	6.5	6.4
($J_{3A,3B}$	−14.3	−14.0	−14.2
Other	Lactoyl 2-H = 4.15 3-H(3H) = 1.21 ($J_{2,3}$ = 7.0)	Mandelyl 2-H = 5.07 Ph = 7.26	Glyceroyl 2-H 4.19 3-H(2H) 3.68 ($J_{2,3}$ = 4.1)
^{13}C NMR spectra			
Assignment δ			
Cysteinyl			
C-1	175.6	172.9	173.2
C-2	56.5	54.0	54.3
C-3	27.3	29.6	25.1
Other	Lactoyl C-1 180.0 C-2 69.9 C-3 21.9	Mandelyl C 1 174.4 C-2 73.2 C-1(Ph) 137.8 C-2,6(Ph) 128.4 C-3,5(Ph) 126.6 C-4(Ph) 126.5	Glyceroyl C-1 174.5 C-2 72.3 C-3 63.3

Proton chemical shifts δ_H (ppm) and coupling constants $J_{Hx,Hy}$ (Hz), and carbon-13 chemical shifts δ_C (ppm) of spectra recorded at 270 MHz and 68 MHz, respectively, of 10 mM (*R*)N-2-hydroxyacylcysteine derivatives (10 mM), pD 1.6.

Materials and methods

N-D-Lactoylcysteine was synthesised by the reaction of S-D-lactoylglutathione (200 mg, 100 mM) with L-cysteine (150 mM) in TRIS/HCl buffer (50 mM), pH 7.4 and 25 °C. The reaction progress was followed by withdrawal of small aliquots of reaction mixture (5 μl) and assay for thiol groups with 5,5′-dithiobis(2-nitrobenzoic acid) (D TNB); as the reaction proceeds, the thiol concentration was expected to increase from 150 to 250 mM. The reaction reached completion after 10 min. Similar reactions were performed with S-L-glyceroylglutathione and S-D-mandelylglutathione and gave N-L-glyceroylcysteine and N-D-mandelylcysteine, respectively. The product mixture was lyophilized to dryness and reconstituted with 10 mM HCl (4 ml). The N-2-hydroxyacylcysteine derivative was separated from residual reactants and reduced glutathione co-product by loading of the product mixture on a column (2.6 cm × 30 cm) of Dowex 50 cation exchange resin, proton form, and elution with 10 mM HCl, 72 ml/h. The eluate was monitored at 254 nm. Residual S-2-hydroxyacylglutathione and cysteine, reduced glutathione and TRIS buffer were retained on the column, and the N-2-hydroxyacylcysteine derivative was eluted in the retention volume range 144–240 ml. The product fractions were lyophilized to dryness to give the N-2-hydroxyacylcysteine derivatives in yields: 77% (N-D-lactoylcysteine), 68% (N-L-glyceroylcysteine) and 63% (N-D-mandelylcysteine). The products were characterized by ^{1}H and ^{13}C NMR spectroscopy (Table 1), FAB mass spectrometry and TLC analysis. The FAB mass spectra of the (*R*)N-2-hydroxyacylcysteine derivatives gave M+1 peaks of m/z 194 (N-D-lactoylcysteine), 210 (N-L-glyceroylcysteine), and 256 (N-D-mandelylcysteine). TLC analysis was on silica gel with developing solvent propan-1-ol:acetic acid:water, in the volume ratio 10:5:5, and detection by ninhydrin in ethanol (0.2%). Chromatographic R_f values were: 0.33 (N-D-lactoylcysteine), 0.52 (N-L-glyceroylcysteine), and 0.58 (N-D-mandelylcysteine).

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