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Reaction of COTC with Glutathione: Structure of the Putative Glyoxalase I Inhibitor

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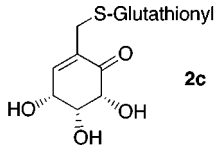
ABSTRACT



2c

The structure of the active glyoxalase I inhibitor derived from the *Streptomyces griseosporeus* metabolite COTC 1 has been conclusively identified by means of total synthesis as 2c. Human glyoxalase I is competitively inhibited by 2c (K_i = 183 ± 6 μM) but is not inhibited by 1 itself.

Cytotoxic methylglyoxal is removed from cells as its glutathione adduct by the enzymes glyoxalase I and II. In 1975, a new inhibitor of glyoxalase I having structure **1** was isolated from the culture broth of *Streptomyces griseosporeus* by Umezawa and co-workers.[1] Known as COTC, compound **1** was also reported to exhibit cytotoxic and cancerostatic activity with low toxicity.[2] The potential of COTC as an anticancer drug has attracted the attention of several synthetic research groups, and five successful total syntheses have now been reported.[3−7] Despite its prospective therapeutic significance, the mechanism of action of COTC remains incompletely understood.

Umezawa et al. noted that by itself COTC had no effect on glyoxalase I, even in the presence of the substrate, methylglyoxal.[1] However, in the presence of reduced glutathione (GSH), time-dependent inhibition of the enzyme was observed. In studies with rat Yoshida glyoxalase I, **1** exhibited an apparent IC_{50} of 8.8×10^{-4} M in phosphate buffer containing methylglyoxal (0.13 M) and GSH (0.04 M). Consequently, the biological activity of COTC was thought to involve nucleophilic addition of GSH, an hypothesis later supported by the change in UV absorbance and loss of titratable SH groups noted when **1** was exposed to GSH in the absence of methylglyoxal.[8] While the reaction product of COTC with GSH has never been isolated and characterized, **1** does react with 2-mercaptoethanol and *p*-bromothiophenol to form thioethers **2a** and **2b**, respectively. Moreover, the structure of **2b** has been confirmed by X-ray crystallography.[2] On the basis of those findings, it has

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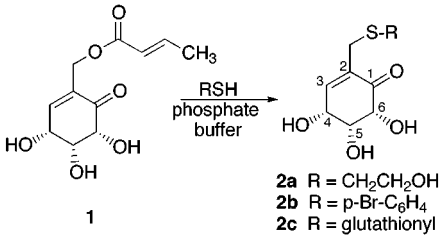
been proposed that GSH combines with **1** to form **2c**. A



variety of mechanisms have been suggested for this transformation, including direct displacement of crotonate,[8] two stepwise 1,4-addition/β-elimination reactions,[9] or 1,4-addition and sigmatropic rearrangement.[8] Such mechanistic speculation seemed premature in the absence of conclusive confirmation of the structure of the GSH reaction product. Here we report the isolation and purification of the GSH adduct of (−)-COTC and subsequent characterization of that structure as **2c**. In addition, we present quantitative kinetic information on the interaction of pure **2c** with glyoxalase I.

Freshly prepared synthetic (−)-**1** (13.5 mg)[7] was stirred with GSH (15.4 mg) in sodium phosphate buffer (pH 7.5) for 10 min at 37 °C and then poured onto an ion-exchange column (2 cm × 2 cm Dowex-1 resin)[10] and eluted with aqueous acetic acid to afford **2c** in 93% yield.[11] Besides the loss of crotonate and gain of glutathionyl resonances, the NMR spectrum of **2c** featured a singlet at δ 6.74 for H3 that is characteristic of β-unsubstituted cyclohexenones such as **1** and its congeners.

Adduct **2c** was a moderately potent competitive inhibitor of human erythrocyte glyoxalase I[12] (K_i = 183 ± 6 μM, Figure 1). Moreover, the inclusion of 0.2 mM COTC **1** in an assay cuvette did not reduce the initial rate of product formation beyond what would be expected from a small increase in the production of additional **2c**,[13] confirming that COTC does not inhibit the enzyme.

The fact that adduct **2c** is a moderately potent competitive inhibitor of glyoxalase I argues against an earlier suggestion

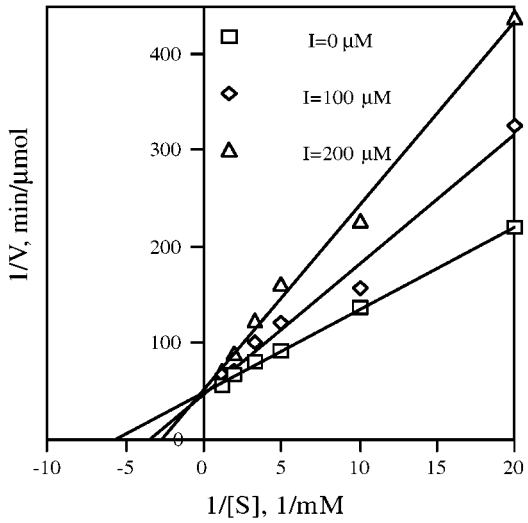


Figure 1. Reciprocal plot of the velocity of the glyoxalase I reaction (ΔA_{240}) versus the concentration of GSH-methylglyoxal thiohemiacetal ([S]) in the presence and absence of **2c** ([I]). In each kinetic run, the concentration of free GSH was maintained at 0.2 mM by varying the total concentration of GSH and methylglyoxal on the basis of the dissociation constant of the hemithioacetal (K_{diss} = 2.2 mM).[15] Conditions: 50 mM phosphate buffer, pH 7.0, 25 °C.

that COTC functions simply by depleting levels of GSH needed to form the methylglyoxal hemithioacetal substrate.[8] Studies with a variety of S-substituted glutathionyl derivatives have established that K_i values decrease with increasing hydrophobicity of the sulfur substituent. These findings suggest the presence of a hydrophobic binding pocket in the active site of human glyoxalase I, which has recently been confirmed using high-resolution X-ray crystallographic analysis.[14] The consequent possibility that less polar analogues of **2c** may bind more tightly to the enzyme will be explored further in ongoing structure−activity studies in our laboratories.

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(11) For **2c**: mp 210−220 °C dec; R_f 0.40 (10:1:5 *n*-PrOH:AcOH:H_2O); ^{1}H NMR (300 MHz, D_2O) δ 6.74 (s, 1 H), 4.51 (dd, 1 H, J = 4.8, 7.0 Hz), 4.43 (m, 2 H), 3.84 (s, 2 H), 3.75 (dd, 1 H, J = 6.4, 5.9 Hz), 3.41, 3.28 (AB q, 2 H, J = 14.0 Hz), 2.95, 2.76 (ABX, 2 H, J_{AB} = 14.0, J_{AX} = 9.1, J_{BX} = 4.8 Hz), 2.48 (m, 2 H, J = 3.8, 3.9, 2.7 Hz), 2.11 (dd, 2 H, J = 7.5, 6.5 Hz); ^{13}C NMR (300 MHz, D_2O) δ 196.3, 172.2, 169.6, 144.3, 130.6, 73.4, 72.5, 64.9, 51.4, 50.2, 39.6, 29.8, 28.5, 26.3, 23.3; MS ES *m*/*z* 464 (M + 1, 57%), 60 (100%).

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(13) The rise in [**2c**] in the assay cuvette was estimated from the second-order rate constant for formation of **2c** from GSH and **1** (k = 0.12 mM^{-1} min^{-1}, pH 7, 25 °C).

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