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Note

Evaluation of dithiobis(thioformate) and *S*-methyl dithiocarbonate as reactive groups for the immobilization of biological molecules

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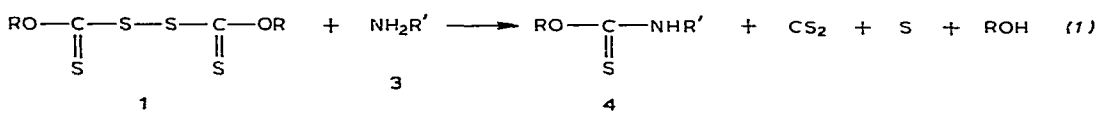
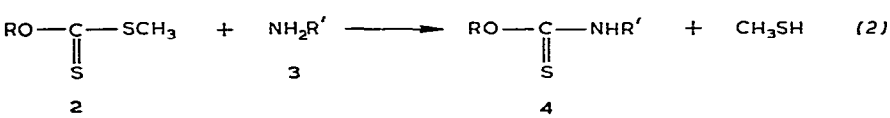
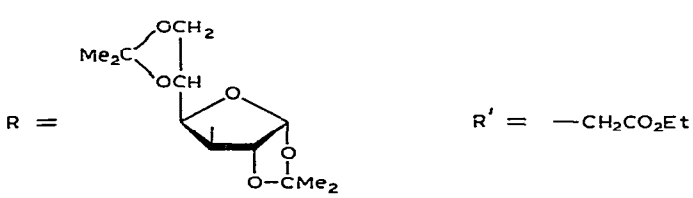
Many methods have been used to immobilize enzymes and other biological molecules by covalent attachment to insoluble matrices[1–5]. Cellulose xanthate has been used to form disulfide linkages with proteins, but necessitates conversion of the protein into a polythiol derivative with *N*-acetylhomocysteine thiolactone[6]. The aim of the present work was to examine the possibility of reaction between a primary amino group, *e.g.*, in a protein or amino acid, and certain xanthate derivatives. Two model xanthate compounds, bis(1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose) 3,3′-dithiobis(thioformate) (**1**) and 1,2:5,6-di-*O*-isopropylidene 3-*O*-[(methylthio)-thiocarbonyl)-α-D-glucofuranose (**2**), were treated with glycine ethyl ester (**3**) under conditions that approximate those suitable for some enzyme immobilizations, *viz.*, in buffer (pH 6.0 or 8.6) at room temperature. Both **1** and **2** reacted readily with **3**, particularly at pH 8.6, to give 3-*O*-ethoxycarbonylmethylthiocarbamoyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**4**) in good yield, indicating the feasibility of the reactions *1* and *2* for coupling enzymes and other biological molecules to polymers.







The pH stability of the thiocarbamate linkage was tested by dissolving **4** (2 mg/100 ml) in aqueous ethanol buffered to the appropriate pH. The absorbance at 241 nm remained constant when such solutions in the pH range 3.6–9.0 were allowed to stand for seven days at room temperature. Experiments involving the coupling of enzyme to cellulose in the above ways are in progress.

EXPERIMENTAL

Melting points were determined on a Leitz microscope heating-stage apparatus and are corrected. Column chromatography and t.l.c. were performed on Silica gel G (Merck) with ethyl acetate–carbon disulphide (9:1) and detection (in t.l.c.) by charring with sulphuric acid. Optical rotations were determined with a Bendix NPL automatic polarimeter. I.r. spectra were recorded with a Pye–Unicam SP 1000 spectrophotometer, u.v. spectra with a Hitachi–Perkin–Elmer model 124 spectrometer, and n.m.r. spectra for solutions in $CDCl_3$ (internal Me_4Si) with a Varian A-60 or a JEOL JNM 4H-100S spectrometer. Chemical-ionization mass spectra were recorded with a GEC-AEI MS-902 spectrometer equipped with an SRIC-CIS-2 chemical ionization source. Ammonia was the ionizing gas.

3-O-Ethoxycarbonylmethylthiocarbamoyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (**4**). — (*a*) To a rapidly stirred solution of **1**[7] (1 g, m.p. 128.5–129.5°) in acetone (50 ml) was added slowly a solution of glycine ethyl ester hydrochloride (1 g) in borate buffer (50 ml, pH 8.6). The mixture was stirred for 1 h at room temperature, filtered, and extracted with ether (3 × 30 ml). The combined extracts were washed with water (3 × 25 ml), dried (Na_2SO_4), and concentrated to yield crude **4** (0.6 g, 99%) that was contaminated (t.l.c.) with **1** and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. Column chromatography of the crude product and recrystallisation from hexane gave **4** (0.227 g, 38%), m.p. 85–86°, $[\alpha]_D^{22}$ −35° (*c* 2.1, chloroform); $\lambda_{max}^{95\%\ EtOH}$ 241 nm (ε_{mM} 12.7); ν_{max}^{KBr} 3300 (NH), 1758 (C=O), and 1560 (Amide II) cm^{-1}. N.m.r. data: δ 1.31 (t, 3 H, J 7.1 Hz, Me), 1.32 (2 s, 6 H, CMe_2), 1.42 and 1.54 (2 s, 6 H, CMe_2), 4.0–4.4 (m, 8 H, H-4,5,6, glycine CH_2, ethyl CH_2), 4.68 (d, $J_{2,1}$ 3.5 Hz, H-2), 5.72 (d, $J_{3,4}$ 1.4 Hz, H-3), 5.87 (d, $J_{1,2}$ 3.6 Hz, H-1), and 6.96 (s, 1 H, NH).

Anal. Calc. for $C_{17}H_{27}NO_8S$: C, 50.37; H, 6.67; N, 3.45; S, 7.90. Found: C, 50.15; H, 6.75; N, 3.18; S, 7.70.

(*b*) To a rapidly stirred solution of **2**[8] (1 g, m.p. 60.5–61.5°) in acetone (50 ml) was added slowly a solution of glycine ethyl ester hydrochloride (1 g) in borate buffer (50 ml, pH 8.6). The mixture was stirred for 12 h at room temperature, filtered, and extracted with ether (3 × 30 ml). The combined extracts were washed with water (3 × 25 ml), dried (Na_2SO_4), and concentrated to yield crude **4** (1.02 g, 88%) that was contaminated (t.l.c.) with **2**. Recrystallisation from hexane gave material (0.447 g, 39%), m.p. 87.5–88°, which was identical to the compound in (*a*).

Chemical-ionization mass spectra of **4** from (*a*) and (*b*) showed major peaks at

m/e 406 ($M + H^+$) and 423 ($M + NH_4^+$). Isotope abundances indicated the presence of one sulphur atom per molecule.

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