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ADDUCTS FROM THE REACTION OF N-ETHYLMALEIMIDE WITH L-CYSTEINE AND WITH GLUTATHIONE

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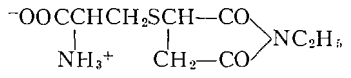
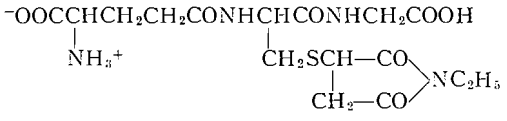
Since the first report of a quantitative reaction between thiols and N-ethylmaleimide (NEM) (1), a number of workers have described the use of NEM as a reagent for the assay of the sulphydryl group (2–6). However, it was only recently that the product from the reaction of L-cysteine and NEM was isolated and characterized as S-(N-ethylsuccinimido)-L-cysteine (I) (7). This note reports an independent isolation of I as well



(I)

as the preparation of S-(N-ethylsuccinimido)-glutathione (II), the adduct from the reaction of NEM with glutathione. Adduct II has not been recorded in any earlier publication.



(II)

The present work was undertaken as part of a project involving the determination of relative amounts of sulphhydryl and disulphide groups in certain proteins in which S^{35} has been incorporated. A suitable carrier for the reaction product between NEM and thiols was sought so that isotope dilution studies may be carried out. With this aim in mind, the behaviors of I and II on acid hydrolysis were studied. Somewhat surprisingly, it was found that after treatment for 18 hours in refluxing 6 *N* hydrochloric acid, I was recovered without change. The products from the hydrolysis of II were identified by paper chromatography as glycine, glutamic acid, and adduct I, exactly as expected for hydrolysis of the peptide bonds with I remaining unaffected. It was also noted that paper chromatographic separation of I from II, cysteine, and cystine can be easily accomplished. These observations indicate that it is likely feasible to use I as a

carrier in isotope dilution studies. If a protein containing S^{35} were allowed to react with NEM and then hydrolyzed in 6 *N* hydrochloric acid, any sulphydryl groups originally present in cysteine residues should give rise to S^{35}-labeled adduct I, which may be isolated with the aid of ordinary I as carrier.

EXPERIMENTAL

*S-(N-Ethylsuccinimido)-*L*-cysteine (I)*

Equal volumes of 0.1 *M* solutions of NEM and L-cysteine hydrochloride were mixed and allowed to stand at room temperature overnight. The resulting solution was concentrated under reduced pressure at 50–60° C. Ethanol was then added to precipitate the product. The latter was dissolved in distilled water, passed through a column packed with the anion exchanger, Dowex 1-x8, eluted with 0.5 *N* acetic acid, concentrated again under reduced pressure, and then crystallized with the addition of ethanol. The yield of purified S-(N-ethylsuccinimido)-L-cysteine, m.p. 194–195° C, decomp., was 85%. It gave a positive ninhydrin reaction and a negative nitroprusside test, which indicated the absence of the sulphydryl group.

Anal. Calc. for $C_9H_{14}O_4N_2S$: C, 43.89; H, 5.73; N, 11.38; S, 13.02. Found: C, 43.89; H, 5.78; N, 11.05; S, 12.90. This procedure for the preparation of I was essentially similar to that reported by Smyth and co-workers (7). However, it was noted that although, when the reactants were mixed, the pH was 1.8, it was not necessary to add sodium hydroxide to the reaction mixture as was done by Smyth *et al.* (7). It was found that inclusion of sodium hydroxide in the reaction mixture often gave a product containing detectable amounts of ash. The elimination of the base and final purification through the Dowex 1-x8 column proved to be quite satisfactory.

S-(N-Ethylsuccinimido)-glutathione (II)

Equal volumes of 0.1 *M* solutions of NEM and glutathione (reduced) were mixed and allowed to stand overnight. The resulting solution was worked up in the same way as in the isolation and purification of I. The product, S-(N-ethylsuccinimido)-glutathione, m.p. 204–206° C, decomp., was obtained in 90% yield. It also gave a positive reaction with ninhydrin and a negative test with nitroprusside.

Anal. Calc. for $C_{16}H_{24}O_8N_4S$: C, 44.44; H, 5.59; N, 12.96; S, 7.42. Found: C, 44.14; H, 5.59; N, 12.88; S, 7.13.

Acid Hydrolysis

One gram of I or II in 20 ml 6 *N* hydrochloric acid was refluxed for 18 hours. The resulting solution was concentrated to a small volume under reduced pressure, passed through a Dowex 1-x8 column, eluted with 0.5 *N* acetic acid, concentrated again and the product crystallized with the addition of acetone. After this treatment, unchanged I was almost quantitatively recovered. That I was the same before and after hydrolysis was established by mixed melting point; unchanged analyses for C, H, N, S; identical infrared absorption spectrum; and behavior in paper chromatography. Ascending paper chromatography on Whatman No. 1 paper with *t*-butanol – water – formic acid (69.5:29.5:1) as solvent was run for 16 hours and developed with 0.1% ninhydrin in acetone. The same spot was obtained for I before and after hydrolysis. On the other hand, paper chromatographic analysis of the hydrolyzate of II gave three spots. By comparison with behaviors of known compounds, these spots were identified as glycine, glutamic acid, and adduct I.

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FINE STRUCTURE IN THE PROTON MAGNETIC RESONANCE SPECTRUM OF 6–6 NYLON

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Correlation of the nuclear magnetic resonance (n.m.r.) spectra of polymers with their physical properties and mechanical behavior has received considerable attention recently and has been reviewed by Slichter (1, 2) and by Sauer and Woodward (3). The existence of a narrow component of the n.m.r. spectrum superimposed on the broad line above certain temperatures has been associated with extensive molecular motion in the amorphous phase of such polymers as branched and straight-chain polyethylene (4–6) and isotactic polypropylene (7, 8). One might anticipate that a narrow component would also be observed in the n.m.r. spectrum of nylon in the vicinity of room temperature, corresponding to the onset in the amorphous part of the nylon of molecular motion which gives rise to energy dissipation and dispersion of elastic modulus in mechanical experiments (3, 9, 10). Although Slichter (11) had already reported the variation with temperature of line width and second moment of nylon 6–6 and given no indication of the existence of fine structure in the spectrum at any temperature, it was decided to reinvestigate this substance. Woodward, Glick, Gupta, and Sauer (12) have published detailed results of the change with temperature of the n.m.r. line width and second moment of a number of polyamides, both thoroughly dried and containing various H_2O and D_2O contents. They do not, however, mention the existence of any fine structure in the spectra. Illers and Kosfeld (13) have recently obtained evidence of a narrow component in the n.m.r. spectrum of a completely dry sample of nylon 6–12.

EXPERIMENTS AND RESULTS

The nylon used in this work was a multifilament commercial yarn containing no delustrant. It was extracted with boiling ether for 8 hours and dried in a vacuum desiccator over phosphorus pentoxide for 24 hours before any experiments were done on it. Initially, this nylon was sealed, without further treatment, into a sample tube under dry nitrogen and its n.m.r. spectrum taken with a broad-line spectrometer of the marginal oscillator type (14), operating at 28 Mc/sec, and a 6-in. Varian electromagnet.

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