The Selective Oxidation of Sulfur-Containing Amino Acids by Diethyl Azodicarboxylate

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Disubstituted azo compounds with electron-attracting substituents[3] are strong electron acceptors.[4] For instance, diethyl azodicarboxylate is capable of dehydrogenating a wide variety of primary or secondary alcohols, mercaptans, and hydrazobenzenes and converting them into aldehydes or ketones, disulfides, and azobenzenes. In this process the hydrogen acceptor is reduced to diethyl hydrazodicarboxylate.

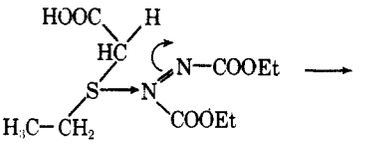
Photochemical and light-independent dehydrogenations with diethyl azodicarboxylates have been carried out.[5,6] The more reactive azo compound 4-phenyl-1,2,4-triazoline-3,5-dione is a superior reagent for the oxidation of alcohols to aldehydes and ketones at room temperature in benzene.[7]

In connection with our work on the selective modification of proteins we studied the oxidation of several sulfur-containing amino acids to sulfoxides with excess diethyl azodicarboxylate at room temperature in various aqueous solvent systems. The azodicarboxylate had to be used in excess, since the aqueous conditions lead to hydrolysis at an appreciable rate with the evolution of nitrogen and carbon dioxide, with some of the azodicarboxylate being reduced to hydrazodicarboxylate, probably by an intermediate diimine. The diethyl hydrazodicarboxylate was removed by extraction with ethyl acetate and the aqueous phase was lyophilized to give high yields of the sulfoxides which were characterized by combustion analysis, infrared spectroscopy, or by analysis on an automatic amino acid analyzer. Table I summarizes our results.

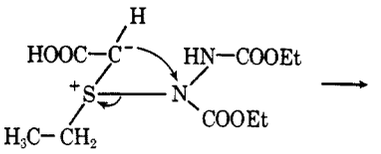
The reaction is dependent on the presence of a proton-donating group, since under similar conditions N-acetyl-L-methionine amide, phenyl sulfide, benzyl sulfide, *n*-hexyl sulfide, and isobutyl sulfide did not react. The oxidation probably proceeds by a reaction mechanism as illustrated below for methionine.

We have also observed that under similar reaction conditions cysteine is either converted into cystine with a slight excess of azodicarboxylate within a few minutes or into cysteic acid with a large excess (400:1) over several days. Tryptophan is also attacked by azodicarboxylate, but the products have so far not been determined.

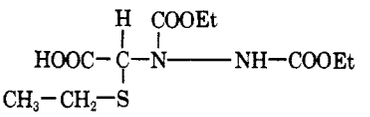
The main product from the reaction of excess diethyl azodicarboxylate (3:1) and ethylthioacetic acid in aqueous acetone for 4 days was a 1:1 adduct (80%), mp 122–125°, for which combustion analysis and nmr spectroscopy were consistent with structure III. This adduct, in analogy to other precedents,[8] is probably formed by an initial attack of the sulfur atom upon the azo grouping I with subsequent rearrangement to the C–N addition product, II → III.



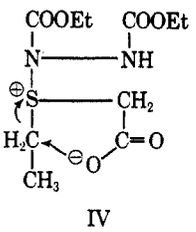
I



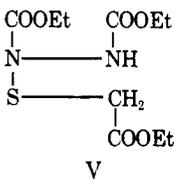
II



III



IV



V

The only other adduct which could have formed by pathway IV → V is ruled out by the nmr data.

Asymmetric oxidation of thioethers to sulfoxides is known in model oxidations[9] as well as in metabolism.[10] To what extent asymmetric induction plays a role in the oxidation of suitable L-methionine derivatives has still to be determined.

Experimental Section

Oxidation of DL-Methionine to DL-Methionine Sulfoxide with Ethyl Azodicarboxylate.—A solution of 1.49 g (10 mmoles) of DL-methionine, 4.74 g (30 mmoles) of ethyl azodicarboxylate,[11] 75 ml of ethanol, and 75 ml of water was stirred at room temperature for 3 hr. During this time the orange color of the solution was discharged and nitrogen and carbon dioxide were evolved.

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(10) L(+)-Methionine sulfoxide occurs in the blowfly (*Phormia regina*): F. Lucas and L. Levenbook, *Biochem. J.*, **100**, 473 (1966).

(11) Ethyl azodicarboxylate was prepared according to the method of S. G. Cohen, R. Zand, and C. Steel, *J. Am. Chem. Soc.*, **83**, 2895 (1961).

TABLE I

Amino acid or derivative	Diethyl azodicarboxylate[a]	Solvent system	Time, hr	% yield of sulfoxide
DL-Methionine	3	Water–ethanol	3	97
L-Ethionine	3	Water–dioxane	1.5	>95
Glycyl-DL-methionine	3	Water–dioxane	1.5	>95
L-Methionine amide·HCl	4	Water–ethanol	4	>98
DL-Methionine methyl ester·HCl	4	Water–methanol	5	>98
S-Ethyl-L-cysteine	18[b]	Water–ethanol	24	>98

[a] Given in the ratio of moles of azodicarboxylate ester *vs.* moles of sulfide. [b] The reagent was added to the reaction mixture of six equal portions during 24 hr.

The solution was concentrated *in vacuo* to a volume of 75 ml and the aqueous mixture was extracted several times with ethyl acetate. The extracts were dried over anhydrous sodium sulfate and evaporated to leave 3.4 g (19.3 mmoles) of ethyl hydrazodicarboxylate, identified by its infrared spectrum.

The aqueous portion was lyophilized to leave 1.61 g (97.5%) of DL-methionine sulfoxide, mp 205–215° dec (lit.[12] mp 220–230° dec). The product was shown to be identical with an authentic sample of DL-methionine sulfoxide by infrared spectroscopy and thin layer chromatography. A sample of the product was placed on an automatic amino acid analyzer and was shown to have the same retention time as authentic DL-methionine sulfoxide.

Oxidation of L-Methionine Amide Hydrochloride.—A solution of 0.92 g (5 mmoles) of L-methionine amide hydrochloride, 3.16 g (20 mmoles) of ethyl azodicarboxylate, 30 ml of ethanol, and 20 ml of water was stirred at room temperature for 4 hr. Thin layer chromatography (silica gel; 70% 1-propanol, 30% water) showed the absence of starting material. The reaction mixture was processed as described for the oxidation of DL-methionine to yield 1.96 g (11.1 mmoles) of ethyl hydrazodicarboxylate and 1.0 g (quantitative yield) of L-methionine amide sulfoxide hydrochloride as a colorless hygroscopic powder. The product was triturated with dry ether, filtered, and dried over phosphorus pentoxide to give a colorless crystalline solid, mp 160–175° dec.

Anal. Calcd for $C_5H_{13}ClN_2O_2S$: C, 29.92; H, 6.53; N, 13.96; S, 15.98. Found: C, 29.88; H, 6.46; N, 13.59; S, 15.81.

The infrared spectrum (KBr) showed a strong peak at 10.0 mμ characteristic of a sulfoxide (solid state).

Oxidation of DL-Methionine Methyl Ester Hydrochloride.—A solution of 0.995 g (5.0 mmoles) of DL-methionine methyl ester hydrochloride, 3.162 (20 mmoles) of ethyl azodicarboxylate, 30 ml of methanol, and 10 ml of water was stirred at room temperature for 5 hr. Processing as described above yielded 1.1 g (quantitative yield) of DL-methionine methyl ester sulfoxide hydrochloride as a colorless viscous oil. The infrared spectrum (film) showed a strong sulfoxide peak at 9.85 mμ.

Oxidation of S-Ethyl-L-cysteine.—A solution of 1.49 g (10 mmoles) of S-ethyl-L-cysteine, 4.74 g (30 mmoles) of ethyl azodicarboxylate, 75 ml of ethanol, and 75 ml of water was stirred at room temperature for 4 hr, during which time the solution became colorless. Thin layer chromatography (silica gel, 70% 1-propanol, 30% water) showed a large amount (*ca.* 80%) of unreacted starting material. Every 4 hr another 30 mmoles of the azo ester was added until a total of 180 mmoles had been added. Thin layer chromatography then indicated the absence of starting material. The solution was concentrated *in vacuo* to 50 ml; distilled water (50 ml) was added and the mixture was extracted with ethyl acetate to remove ethyl hydrazodicarboxylate.

The aqueous portion was lyophilized to leave 1.66 g (quantitative yield) of S-ethyl-L-cysteine sulfoxide as a colorless powder, mp 140–150°. Recrystallization from aqueous ethanol gave colorless crystals, mp 157–159° dec.

Anal. Calcd for $C_5H_{11}NO_3S$: C, 36.35; H, 6.71; N, 8.48. Found: C, 36.57; H, 6.66; N, 8.34.

Reaction of Ethylthioacetic Acid with Ethyl Azodicarboxylate.—A solution of 1.20 g (10 mmoles) of ethylthioacetic acid, 4.74 g (30 mmoles) of ethyl azodicarboxylate, 30 ml of acetone, and 10 ml of water was stirred at room temperature for 4 days, after which time the orange solution had become colorless. The solution was concentrated to 10 ml, diluted with a solution of 2.52 g (30 mmoles) of sodium bicarbonate in 30 ml of distilled water, and the mixture was extracted with ethyl acetate to remove ethyl hydrazodicarboxylate.

The aqueous phase was acidified with dilute hydrochloric acid to pH 2 and extracted with chloroform. The extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated to yield 2.35 g (80%) of the 1:1 adduct III as a crystalline solid. Recrystallization from water gave colorless prisms, mp 122–125°.

Anal. Calcd for $C_{10}H_{18}N_2SO_6$: C, 40.80; H, 6.17; N, 9.52; S, 10.89. Found: C, 40.96; H, 6.02; N, 9.43; S, 10.94.

The nmr spectrum ($CDCl_3$) showed a triplet centered at 1.32 (9 protons), a quartet centered at 2.85 (2 protons), a quartet centered at 4.30 (4 protons), a singlet at 6.08 (1 proton), a broad band at 7.35 (1 proton), and a singlet at 9.38 (1 proton) ppm.

Attempted Oxidation of Certain Thioethers with Ethyl Azodicarboxylate.—Attempts were made to oxidize N-acetyl-L-methionine amide, *n*-hexyl sulfide, isobutyl sulfide, and benzyl sulfide with excess ethyl azodicarboxylate in ethanol–water or acetone–water solution at room temperature and at reflux. In all cases only the starting thioether and ethyl hydrazodicarboxylate were isolated.

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Photochemical Rearrangement of a γ,δ-Cyclopropyl-α,β-Unsaturated Ketone

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One of the remaining problems concerning conjugated cyclopropyl systems is establishing the factors involved in determining which bond of the cyclopropane ring will undergo cleavage during the course of a photochemical reaction. From a consideration of only the bicyclo[3.1.0]hexan-2-one system (**1**), the situation appears quite simple. Several reports have indicated that derivatives of **1** undergo predominant or exclusive cleavage of the external bond a rather than the internal bond b, to afford products of type **2**.[1] This is not surprising since, within the rigid skeleton of **1**, it is the orbitals of bond a which are more nearly parallel to, and can more effectively overlap with, the π orbitals of the carbonyl group. However, the introduction of a double bond to give the bicyclo[3.1.0]hex-3-en-2-one system **3** dramatically alters the photochemical behavior; derivatives of **3** undergo almost exclusive cleavage of the internal bond b to afford products ultimately derived from an intermediate of type **4**.[2] Parallel behavior is exhibited by the bicyclo[4.1.0]hept-4-en-2-one analog **5**, which undergoes cleavage to **6**[3]

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