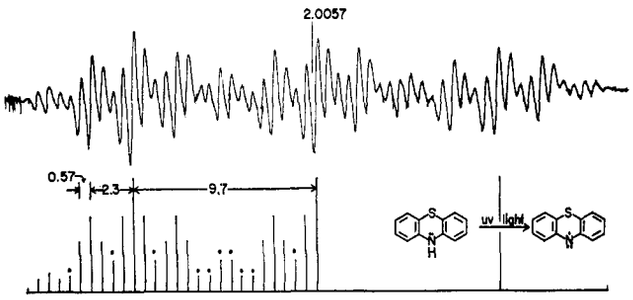

Figure 1.—The esr spectrum of a solution of phenothiazine in 95% ethanol after irradiation with ultraviolet light. The vertical line (g = 2.0057) represents the center of the spectrum of the nitrosodisulfonate ion. The line diagram in the lower half of the spectrum was constructed with the hyperfine spacings of the experimental spectrum and the intensities of a 1:1:1 nitrogen triplet split successively into two quintets. The small circles above some lines indicate the overlap and summation of two lines.

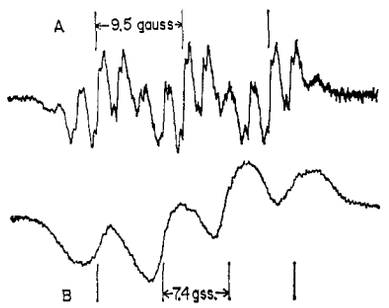

Figure 2.—The esr spectrum of a solution of phenothiazine in absolute ethanol after irradiation with ultraviolet light (A). The esr spectrum of a similar, irradiated solution after dilution with an equal volume of alcoholic sulfuric acid (10 volumes of sulfuric acid to 90 volumes of absolute ethanol) (B).

tion of the II which had been formed by the irradiation of I. This is the reverse of the observation of Lewis and Bigeleisen, and is expected from the higher acidity of our final solutions.

Experimental Section

Phenothiazine, practical grade, Distillation Products Industries, was recrystallized from butanol and had mp 182–184°. No impurity was detected with the use of thin layer chromatography.

Irradiations were carried out on solutions in quartz esr cells or silica cuvettes. In some irradiations a G.E. H250-A5 lamp was used. In others a Dallons Laboratory low-intensity lamp was used, similar to a mineralight lamp, with the major emission at 2537 A.

Esr spectra were obtained with a Varian Associate's instrument using a dual-sample cavity.[9]

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The Oxidation of Selenocystamine to 2-Aminoethaneselenenic Acid

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The rapid conversion of diselenides to seleninic acids by oxidizing agents without the isolation or even

$$\mathrm{RSeSeR} \xrightarrow[\mathrm{H_2O}]{[\mathrm{O}]} 2\ \mathrm{RSeO_2H}$$

detection of intermediate compounds leaves uncertainty as to the steps by which the above-mentioned oxidation takes place. Fromm and Martin[2] attempted to oxidize dibenzyl diselenide to dibenzyl diselenoxide but did not obtain the desired product. Twiss[3] likewise was unsuccessful in obtaining a compound of an intermediate oxidation state by the action of hydrogen peroxide on dibenzyl diselenide. In the ozonolysis of dialkyl and diaryl diselenides to seleninic anhydrides, Ayrey, Barnard, and Woodbridge[4] were unable to isolate intermediate products. They suggested, however, that the following may be the sequence by which a diselenide is converted into a seleninic acid by either ozone or *t*-butyl hydroperoxide.

$$\mathrm{RSeSeR} \longrightarrow \mathrm{RSeSeR} \overset{\|}{\mathrm{O}} \longrightarrow \mathrm{RSeOSeR} \longrightarrow \mathrm{RSeOSeR} \overset{\|}{\mathrm{O}} \longrightarrow$$

$$\mathrm{RSeOSeR} \quad \overset{\|}{\mathrm{O}}\ \overset{\|}{\mathrm{O}}$$

$$\downarrow \mathrm{H_2O}$$

$$2\mathrm{RSeO_2H}$$

Caldwell and Tappel[5] attribute the failure to observe a diselenoxide in the oxidation of selenocystine to the probable instability and high reactivity of the former type of compound.

Cystamine [bis(2-aminoethyl) disulfide] can be oxidized stepwise to the monoxide, 2-aminoethyl 2-aminoethanethiolsulfinate,[6] the dioxide, 2-aminoethyl 2-aminoethanethiolsulfonate,[7] before 2-aminoethanesulfinic acid[8] is obtained.

In a recent communication[9] we reported that selenocystamine [I, bis(2-aminoethyl) diselenide] and its dihydrochloride salt are oxidized by excess hydrogen peroxide to 2-aminoethaneseleninic acid (H_2NCH_2-CH_2SeO_2H). In an effort to isolate selenium compounds related to the above series of aminoethyl-substituted sulfur intermediates, compound I dihydrochloride was subjected to oxidation with 1 equiv of *m*-chloroperbenzoic acid in a method analogous to the preparation of 2-aminoethyl 2-aminoethanethiolsulfinate dihydrochloride.[6b] Instead of the desired monoxide there was obtained 47% of the starting material and 43% of a new compound, $C_2H_8ClNOSe$ (II hydrochloride). Use of 2.2 equiv of *m*-chloroperbenzoic acid raised the yield of II hydrochloride to 96.9%. This material could be prepared in a simpler manner and in 98% yield by the action of 2.2 equiv of hydrogen peroxide on an aqueous solution of I dihydrochloride.

Oxidation of I (free base) with 2.2 equiv of hydrogen peroxide resulted in the formation of a crystalline material which gave microanalytical data for C_2H_7NOSe.

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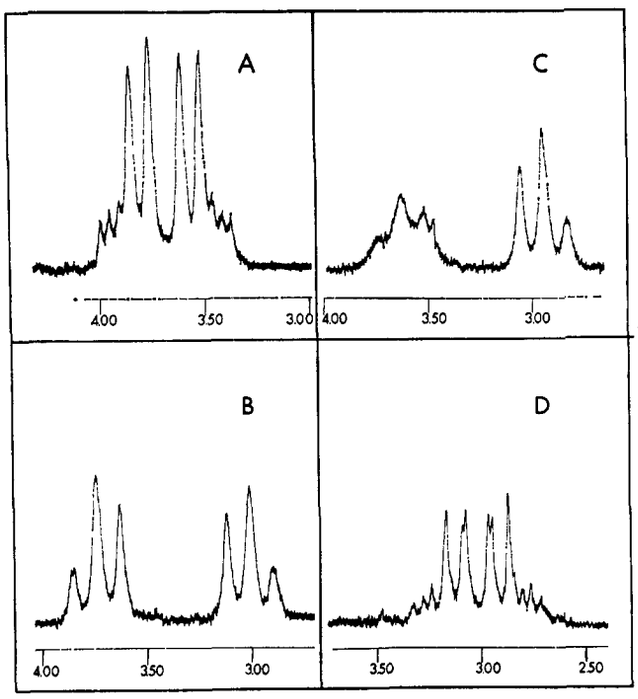

Figure 1.—Nmr spectra in D_2O with tetramethylsilane in CCl_4 as external reference: (A) 2-aminoethaneselenenic acid (II) hydrochloride at pH 2.5; (B) solution adjusted to pH ~7.5; (C) solution adjusted to pH ~8.0; (D) solution adjusted to pH ~11.0. The scale is in ppm from the reference.

A molecular weight determination indicated that the empirical and molecular formulas were identical. Compound II could be converted quantitatively to its hydrochloride salt by the addition of hydrochloric acid to pH 2.5 followed by the evaporation of the water at reduced pressure. Treatment of II with excess hydrochloric acid resulted in extensive decomposition of the product on taking the solution to dryness.

An aqueous solution of II is essentially neutral (*ca.* pH 7.4) suggesting an amino acid like structure for the compound. The selenenic acid structure below is proposed for compound II.

$$H_2NCH_2CH_2SeOH$$
II

The nuclear magnetic resonance spectrum of II hydrochloride in D_2O at its natural pH showed a typical A_2B_2 spectrum[10] (Figure 1A) centered at δ 3.69 due to the two pairs of methylene protons. Any coupling from the amino hydrogens is removed by complete isotopic exchange. The pH of the solution was increased gradually by the addition of small increments of sodium deuterioxide in D_2O. At pH *ca.* 7.5 the nmr spectrum took on the appearance of two triplets (Figure 1B) each with a spacing of 7 cps and centered at δ 3.01 and 3.74, respectively, which is attributed to the existence of the molecule in the zwitterionic form ($D_3N^+CH_2CH_2SeO^-$). The 7-cps coupling is typical of first-order coupling by rotationally averaged methylene protons.[11] The low-field triplet is assigned to the methylene group adjacent to the nitrogen atom since the positive charge would deshield these protons. Increasing the pH to *ca.* 9 afforded an nmr spectrum (Figure 1C) in which the low-field triplet is subject to uncertainty broadening as deuterons are removed from the adjacent quaternary amino group. At pH *ca.* 11 abstraction of deuterons from the amino group was complete and since the deshielding effect of the positive charge of the quaternary amino group observed at pH *ca.* 7.5 was now removed, the spectrum was compressed into a second A_2B_2 spectrum (Figure 1D) centered at δ 3.01 and was accordingly assigned to $D_2NCH_2CH_2SeO^-$. Rapid acidification of the solution at this point with a solution of hydrogen chloride in deuterium oxide caused II hydrochloride to be reformed whose nmr spectrum is identical (Figure 1A) with the spectrum of the original II hydrochloride. The nmr data are, therefore, in agreement with the proposed structural assignment for II.

Reduction of II hydrochloride with 0.5 equiv of hydrazine gave I dihydrochloride in an 80% yield. Use of sodium borohydride on II (free base) was less satisfactory inasmuch as the reduction tended to proceed beyond the diselenide stage to the selenol.

$$HCl\cdot H_2NCH_2CH_2SeOH + 0.5H_2NNH_2 \longrightarrow 0.5(HCl\cdot H_2NCH_2CH_2Se-)_2 + 0.5N_2 + H_2O$$

Treatment of II hydrochloride with 1 equiv of hydrogen peroxide converted only about half of the material to 2-aminoethaneseleninic acid.

2-Aminoethaneselenenic acid is the first example of a stable aliphatic selenenic acid. Those few aromatic selenenic acids that are known[12] are said to exhibit greater stability than the analogous sulfenic acids.[13] It should be mentioned that the corresponding sulfur compound, 2-aminoethanesulfenic acid, is, to our knowledge, unknown.

Experimental Section[14]

Oxidation of I Dihydrochloride with *m*-Chloroperbenzoic Acid.—A solution of 4.46 g (0.022 mole) of 85% *m*-chloroperbenzoic acid in 15 ml of methanol was added over 1 hr to a solution of 3.19 g (0.01 mole) of I dihydrochloride. The solution was permitted to stand for 1 hr and was evaporated to dryness under reduced pressure. The residue was extracted with water and the filtered extract was evaporated to dryness under reduced pressure. The syrupy residue was triturated with isopropyl alcohol to give 3.4 g (96.9% yield) of 2-aminoethaneselenenic acid hydrochloride, mp 113–115° dec. Recrystallization of the compound from methanol served to remove a small amount of yellow color but did not alter the melting point.

Anal. Calcd for $C_2H_8ClNOSe$ (176.51): C, 13.61; H, 4.56; N, 7.93; Se, 44.73; Cl, 20.09. Found: C, 13.62; H, 4.87, N, 7.79; Se, 44.55; Cl, 19.87.

Oxidation of I Dihydrochloride with Hydrogen Peroxide.—To a stirred and ice-cooled solution of 6.38 g (0.02 mole) of I dihydrochloride in 20 ml of water was added dropwise over 1 hr 5 ml (0.044 mole) of 30% hydrogen peroxide diluted with an equal volume of water. The solution was permitted to stand overnight at room temperature and evaporated to dryness on a rotary evaporator under reduced pressure at 50°. The syrupy residue was triturated with isopropyl alcohol and cooled to afford 6.98 g (99.4% yield) of II hydrochloride, mp 113–115° dec.

Oxidation of I (Free Base) with Hydrogen Peroxide.—To a magnetically stirred solution of I (3.67 g, 0.015 mole) in 10 ml

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(14) Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Microanalyses were performed by Mr. Joseph F. Alicino, Metuchen, N. J. Nmr spectra were taken on a Varian A-60 spectrometer.

of water was added dropwise 3.96 ml (0.033 mole) of 30% hydrogen peroxide in 5 ml of water, the temperature being maintained below 25°. After standing for 1 hr the resultant solution was evaporated at 50° at reduced pressure and the semisolid residue that was obtained was triturated with isopropyl alcohol affording 3.32 g (80.8% yield) of off-white crystals, mp 125–128° dec, of 2-aminoethaneselenenic acid. This compound is difficult to recrystallize and decomposes after several months of storage.

Anal. Calcd for C_2H_7NOSe (140.04): C, 17.15; H, 5.03; N, 10.00; Se, 56.38. Found: C, 17.45; H, 4.52; N, 9.44; Se, 56.77; mol wt, 131.[15]

Reduction of II Hydrochloride with Hydrazine.—To a solution of 1.76 g (0.01 mole) of II hydrochloride in 40 ml of methanol was added dropwise 0.22 g of 95% hydrazine. The solution was left overnight at room temperature and then evaporated to dryness on a steam bath with the aid of a stream of nitrogen. The residue was triturated with a small quantity of cold ethanol and filtered to give 1.27 g (79.6% yield) of I dihydrochloride, mp 188–189° (lit. 188°,[16] 177–179° dec,[17] 186–188°[9]).

Nmr Spectral Determinations.—The pH dependence of the nmr spectrum of II was studied in the following manner. About 0.1-ml portions of NaOD in D_2O were added to a solution of II hydrochloride in D_2O. The nmr spectrum was scanned after each addition of base from the natural pH of II hydrochloride to *ca.* pH 11. After the last scan the solution was returned to pH 3 with hydrogen chloride in D_2O. The nmr spectrum obtained was identical with that obtained from the starting II hydrochloride.

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Amino Acids and Peptides. V.[1] Synthesis of the C-Terminal Tripeptide Sequence (A_{27}–A_{29}) of Glucagon

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Several paths have been described for the preparation of the C-terminal tetrapeptide and related portions of the hyperglycemic hormone glucagon.[2] The earliest scheme disclosed the synthesis of N-phthaloyl-L-leucyl-L-methionyl-L-asparaginyl-O-*t*-butyl-L-threonine *t*-butyl ester by stepwise use of a series of *p*-nitrophenyl esters.[3] Alternatively, a potential condensation involving N-trityl-L-leucyl-L-methionine and L-asparaginyl-L-threonine methyl ester was abandoned owing to difficulties in the hydrolysis of the corresponding N-trityl methyl ester derivative. A second major route utilized an azide sequence between N-phthaloyl-L-leucyl-L-methionyl hydrazide and L-asparaginyl-L-threonine *t*-butyl ester as well as a mixed anhydride reaction with phthaloyl-L-leucyl-L-methionine and L-asparaginyl-L-threonine *t*-butyl ester to give the same tetrapeptide fragment.[4] In a third but incomplete work, N-ethylbenzisoxazolium fluoroborate was employed as the activation agent at various stages.[5] Finally, the tripeptide moiety has been obtained in the form of both N-1-methyl-2-benzoyl-vinyl-L-methionyl-L-asparaginyl-L-O-*t*-butyl-L-threonine *t*-butyl ester and the equivalent N-trifluoroacetyl-substituted derivative.[6] The former compound was prepared from a mixed anhydride reaction between N-1-methyl-2-benzoyl-vinyl-L-methionine and L-asparaginyl-O-*t*-butyl-L-threonine *t*-butyl ester, while the latter product was constructed through an azide coupling with N-trifluoroacetyl-L-methionine hydrazide and the same dipeptide.

A new variant for the protected tripeptide sequence of glucagon is reported here. N-benzyloxycarbonyl-O-*t*-butyl-L-threonine *t*-butyl ester (I) was reduced to O-*t*-butyl-L-threonine *t*-butyl ester (II), which was condensed with N-benzyloxycarbonyl-L-asparagine (III) with the aid of 2-ethyl-5-phenyloxazolium-3′-sulfonate (reagent K)[7] to give N-benzyloxycarbonyl-L-asparaginyl-O-*t*-butyl-L-threonine *t*-butyl ester (IV). This step avoids the need for N-benzyloxycarbonyl-L-asparagine *p*-nitrophenyl ester and gives a better overall yield of compound IV. Hydrogenolysis of the dipeptide IV produced L-asparaginyl-O-*t*-butyl-L-threonine *t*-butyl ester (V), which on reaction with the 2,4,5-trichlorophenyl ester[8] of N-benzyloxycarbonyl-L-methionine (VI) afforded crystalline N-benzyloxycarbonyl-L-methionyl-L-asparaginyl-O-*t*-butyl-L-threonine *t*-butyl ester (VII). The tripeptide VII was formed in lower yield by combining the dipeptide amine V with N-benzyloxycarbonyl-L-methionine in the presence of reagent K. Removal of the protecting benzyloxycarbonyl group of compound VII with excess 30% palladium-on-carbon catalyst slowly yielded the corresponding oily amine (VIII).

The electrophoretic pattern of glucagon on starch shows two characteristic compounds present in the approximate ratio 9:1. If the slower moving, major component is separated and again subjected to electrophoresis, the identical fractions reappear in the same proportion. The contaminant has about half the biological activity and *qualitatively* contains the same amino acids, suggesting that the faster moving material is derived from glucagon.[9] These observations could be attributed to a conversion of the methionine residue in glucagon by air oxidation to the equivalent sulfoxide analog. The reduced physiological activity of this latter product may be associated with steric incompatability at a specific receptor site. In order to test this assumption at a future date, it would be desirable to incorporate the sulfoxide residue into synthetic glucagon. Accordingly, the tripeptide VII was oxidized with dilute hydrogen peroxide to produce the corresponding N-benzyloxycarbonyl-L-methionyl sulfoxide L-asparaginyl-O-*t*-butyl-L-threonine *t*-butyl ester (IX). The sulfoxide IX on thioglycolic acid treatment was easily reconverted into the parent compound VII with no loss in optical activity. This last sequence was patterned on a procedure elaborated for methionine ↔ methionine sulfoxide interconversions.[10]

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