[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA]

The Reaction of Myleran with L-Cysteine Ethyl Ester[1,2]

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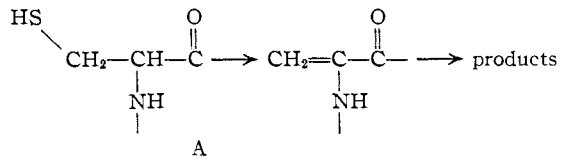
The reaction of 1,4-dimethanesulfonoxybutane (myleran) (I) with two equivalents of L-cysteine ethyl ester (II) in ethanol has been studied, and the following products have been identified: (a) tetrahydrothiophene (III), (b) 2-methyl-2,4-carbethoxythiazolidine and (c) alanine 3,3'-(tetramethylenedithio)-bis-diethyl ester. The unique feature of this reaction is the *in vitro* "sulfur stripping or depleting" action of the bis-alkylating agent, and it is suggested that this reaction may be responsible for the physiological activity of the bis-alkylating agents.

It has been shown in recent years that a variety of alkylating agents are active in inhibiting tumor growth.[3,4a] The selectivity of these alkylating agents for sulfhydryl groups *in vivo*,[5] the impressive evidence of the importance of sulfhydryl compounds in cell division and tumor growth,[4b] and the relationship of cysteine, its precursors[6] and glutathione[4b] to leukocyte metabolism and leukemia all suggest that the mechanism of action of these bis-alkylating agents in chemotherapy may involve their reaction with the sulfhydryl group of proteins or other cell metabolites. However, there is no agreement among the workers in the field as to the mechanism of action of these alkylating agents as chemotherapeutic agents against malignant disease. Alexander,[7] for example, has presented strong arguments in favor of the view that the alkylating agents act by reaction with the phosphate groups of nucleic acids, although this opinion has been disputed by others.

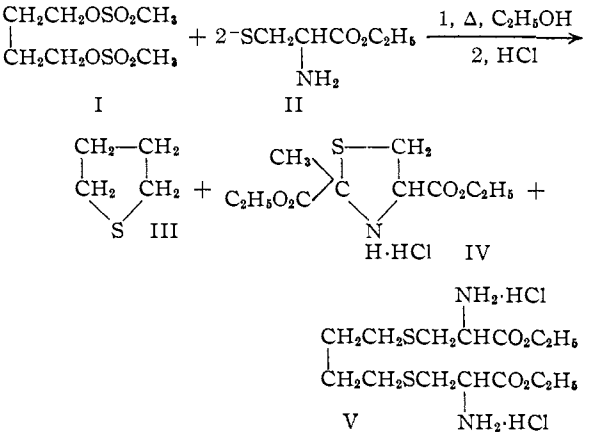
The rather wide range of bis-alkylating agents effective in varying degrees in inhibiting tumor growth, and specifically in treating leukemia, militates against the hypothesis that the formation of antimetabolites is responsible for their action. Our results suggest that the effectiveness of the bis-alkylating agents may be related to their ability to remove or "strip" sulfur from groups such as A.



A

We now wish to report such a reaction, involving the sulfhydryl group of L-cysteine ethyl ester, and 1,4-dimethanesulfonoxybutane.

The reaction of 1,4-dimethanesulfonoxybutane (myleran) (I) with two equivalents of the sodium salt of L-cysteine ethyl ester (II) in ethanol has been shown to give a complex mixture from which three principal products (III, IV, V) have been isolated.



Tetrahydrothiophene (III) was isolated from the ethanol distillate of the reaction mixture as the mercuric chloride salt[8] (11.9% yield).

An ether solution of the reaction residue, obtained subsequent to removal of solvent ethanol, was adjusted to *p*H 3 by the addition of saturated ethereal hydrogen chloride, and the precipitate of V (21.8% yield, melting at 158–163° dec.) was collected and recrystallized from ethanol–ether (V, m.p. 167–168° dec., 14.5% yield, pure).

The ether solution, from which V has been removed, was treated with additional saturated ethereal hydrogen chloride and the precipitate of IV (15.5% yield, m.p. 130–135°) was collected and recrystallized from ethanol–ether (IV, 7.3% yield, m.p. 138–139°). Compound IV gave a positive nitroprusside test and reacted with benzyl chloride in aqueous carbonate solution to give S-benzylcysteine[9] (11.8% yield). Compound IV was shown to be 2-methyl-2,4-carbethoxythiazolidine hydrochloride by its independent synthesis (40.2% yield, m.p. 137–140°) from ethyl pyruvate and cysteine ethyl ester hydrochloride.

The formation of compounds III and IV is tentatively explained, as summarized by the reaction sequence shown.

The remaining product (*ca.* 50% yield) has not been characterized, but is thought to be a mixture of products derived by further reaction of either/or III, IV, V and ammonia with myleran. A more detailed study of the reactions of such bis-alkylating agents with cysteine, cystine, methionine, glutathione and polypeptides containing cysteine and methionine is in progress.

(1) This investigation was supported by the National Cancer Institute, National Institutes of Health, Grant CY 3907.

(2) Presented at the 136th National Meeting of the American Chemical Society, Atlantic City, N. J., September, 1959.

(3) *Cf.* W. C. J. Ross, *Ann. New York Acad. Sci.*, **68**, 669 (1958); K. A. Stacey, M. Cobb, S. F. Cousens and P. Alexander, *ibid.*, **68**, 682 (1958); E. L. Powers and J. H. Pomeroy, *ibid.*, **68**, 702 (1958).

(4) (a) *Cf.* W. Dameshek and F. Gunz, "Leukemia," Grune and Stratton, New York, N. Y., 1958. (b) *Cf.* W. Dameshek and F. Gunz, *ibid.*, pp. 103–104.

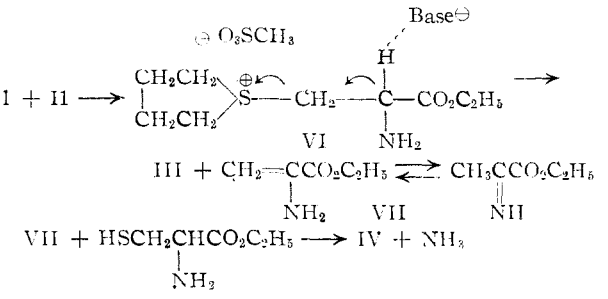
(5) *Cf.* J. J. Roberts and G. P. Warwick, *Ann. New York Acad. Sci.*, **68**, 722 (1958).

(6) *Cf.* A. S. Weisberger, L. G. Suhrland and J. Seifter, *Blood*, **11**, 1 (1956); A. S. Weisberger and L. G. Suhrland, *ibid.*, **11**, 18 (1956).

(7) P. Alexander, *Nature*, **169**, 226 (1952).

(8) E. V. Whitehead, R. A. Dean and F. A. Fidler, THIS JOURNAL, **73**, 3634 (1951).

(9) *Cf.* S. Ratner and H. T. Clarke, *J. Am. Chem. Soc.*, **59**, 200 (1937).



Experimental[10]

Reaction of L-Cysteine Ethyl Ester Hydrochloride with Myleran.—A solution of L-cysteine ethyl ester hydrochloride (42.7 g., 0.23 mole) in 500 ml. of absolute ethanol was added to a solution of sodium ethoxide, prepared by treating sodium (11.5 g., 0.50 mole) with 200 ml. of absolute ethanol. The resulting suspension was added rapidly to a hot solution of myleran (27.0 g., 0.11 mole) in 1700 ml. of absolute ethanol. The reaction mixture was heated at reflux for 2.5 hours and after cooling (ice-bath), the solid which precipitated was removed by filtration and washed twice with 200 ml. of ethanol. The washes and filtrate were combined, and the ethanol was removed under reduced pressure on a steam-bath. Any further precipitate which formed was removed by filtration and washed with ethanol.

Isolation of Tetrahydrothiophene.—Tetrahydrothiophene (III) was isolated from the ethanol distillate of the reaction mixture as the mercuric chloride salt.[8] A solution of mercuric chloride (16.3 g., 0.06 mole) in 200 ml. of ethanol was added to the ethanol distillate. The mixture was allowed to stand at 0° for one week. The resulting solid was collected and washed with ethanol, affording 4.0 g. (10.1%) of colorless needles melting at 128–130°. An additional 0.7 g. melting at 126–128° was obtained by concentrating the supernatant liquors to 200 ml. Recrystallization of the combined product from ethanol gave 2.5 g. (6.3%) of material which melted at 128–130°; mixture m.p. with authentic material,[8] m.p. 128–130°, was 128–130°.

Isolation of Alanine, 3-3′-(Tetramethylenedithio)-bis-, Diethyl Ester, Dihydrochloride.—Dry ether (200 ml.) was added to the residual yellow oil which remained after removal of the ethanol solvent. The ether solution was filtered to remove a gummy precipitate, and the ether was removed from the filtrate under reduced pressure on a water-bath to leave 30 g. of a yellow liquid. This product was dissolved in 100 ml. of dry ether, and saturated ethereal hydrogen chloride was added dropwise with stirring and cooling until *p*H 3 (*p*H paper) was reached. About 140 ml. of ethereal hydrogen chloride was required. The precipitate of V which formed (10.7 g., 21.8% yield, melting at 158–163° dec.) was collected, washed with ether and recrystallized from ethanol–ether to give 7.2 g., 14.5% yield, of product melting at 167–168° dec. Further recrystallization of this product from ethanol–ether gave a sample melting at 168–169° dec.

Anal. Calcd. for $C_{14}H_{30}N_2S_2Cl_2O_4$: C, 39.53; H, 7.11; N, 6.59; S, 15.07. Found: C, 39.26; H, 7.02; N, 6.47; S, 15.16.

Isolation of 2-Methyl-2,4-carbethoxythiazolidine Hydrochloride.—The ether solution from which V had been removed was treated with additional saturated ethereal hydrogen chloride, and the precipitate of IV, which formed after the mixture had stood in the refrigerator several hours, was collected and washed with ether to yield 5.1 g. (15.5%) of crystals which melted at 130–135°. Recrystallization of this product from ethanol–ether gave 2.3 g. (7.3%) of colorless crystals melting at 138–139°. A sample prepared by further recrystallization of this product from ethanol–ether melted at 138.5–140°.

Anal. Calcd. for $C_{10}H_{18}NSClO_4$: C, 42.33; H, 6.39; N, 4.94; S, 11.30. Found: C, 42.31; H, 6.44; N, 4.90; S, 11.59.

Proof of Structure of IV.—Compound IV gave a positive nitroprusside test and reacted with benzyl chloride in aqueous carbonate solution to give s-benzylcysteine.[9] A mixture of compound IV (2.3 g., 0.0081 mole), benzyl chloride (1.3 g., 0.01 mole) and potassium carbonate (5.5 g., 0.04 mole) in 20 ml. of water was heated at reflux with stirring for 5 hours. The reaction mixture was cooled to room temperature and extracted twice with 10 ml. of chloroform. The aqueous layer was cooled (ice-bath) and adjusted to *p*H 7 (*p*H paper) with 2 *N* hydrochloric acid. The product which separated was collected and recrystallized from water. The product was then washed with water and finally with ethanol; it then weighed 0.2 g. (11.8%) and melted at 209–210° dec. Further recrystallization of this material from water gave a sample melting at 210–211° dec.; reported melting point 215–216° dec., corrected.

Compound IV was shown to be 2-methyl-2,4-carbethoxythiazolidine hydrochloride by comparison of its physical properties with a sample prepared by an independent synthesis.

2-Methyl-2,4-carbethoxythiazolidine Hydrochloride.—A solution of ethyl pyruvate (14.6 g., 0.12 mole) and L-cysteine ethyl ester hydrochloride (16.8 g., 0.09 mole) in 300 ml. of ethanol was heated at reflux for 6 hours. The solvent was removed under reduced pressure on the steam-bath, and 250 ml. of dry ether was added to the residual oil. The product was cooled at 0° for two days; the crystals which formed were collected, washed with ether, and recrystallized from ethanol–ether affording 9.2 g. (36%) of colorless crystals melting at 137–140°. An additional 1.1 g. of product melting at 136–139° was recovered from the supernatant liquors by concentration and recrystallization. Further recrystallization of the combined product gave 6.0 g. (23.4%) of pure crystals melting at 139–141°, mixture m.p. with compound IV, 138.5–141°. Compound IV and the synthetic material just described had identical infrared spectra.

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(10) All melting points are uncorrected.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

Synthesis of a Biologically Active Analog of Oxytocin, with Phenylalanine Replacing Tyrosine[1,2]

BY MIKLOS BODANSZKY AND VINCENT DU VIGNEAUD

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2-Phenylalanine oxytocin is an analog of oxytocin in which the tyrosyl residue of the hormone is replaced by phenylalanyl. The compound has been synthesized by two methods which were previously used for the synthesis of oxytocin. The analog was tested for uterine-contracting, avian depressor and milk-ejecting activity. It exhibited activity in these tests, but not to the extent shown by oxytocin. Thus the phenolic hydroxyl group contributes strongly to the potency of the hormone but is not essential qualitatively to its activity with respect to the biological properties so far tested.

In a series of studies on the relation of structure to biological activity of oxytocin an analog of this hormone was synthesized. This analog is different from oxytocin only to the extent that the

(1) This work was supported in part by a grant from the National Heart Institute, U. S. Public Health Service, Grant No. H 1675.

(2) A preliminary report of this work has appeared [M. Bodanszky and V. du Vigneaud, THIS JOURNAL, **81**, 1258 (1959)].