## [CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

# CHEMICAL REACTIONS OF THE NITROGEN MUSTARD GASES.<sup>1</sup> II. THE COMPOSITION OF AGED UNBUFFERED SOLUTIONS OF METHYL-BIS(β-CHLOROETHYL)AMINE

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## Received March 22, 1946

It has been found (1, 2, 3) that, upon standing at room temperature for 24 hours or longer, 1% aqueous solutions of methyl-bis( $\beta$ -chloroethyl)amine (MBA) exhibit a neurotoxic action upon administration to experimental animals. The present investigation was undertaken to determine the nature of the transformation products of MBA responsible for this pharmacological action.

Hartley, Powell, and Rydon (1) studied the composition of 1% solutions of MBA aged for 48 hours and found, by fractionation of picrates, that the solutions contained 25% of the dichloro cyclic dimer [N,N'-dimethyl-N,N'-bis- $(\beta$ -chloroethyl)piperazinium dichloride (V)<sup>4</sup>], 15% unchanged MBA, and 35% of the chlorohydrin [methyl- $\beta$ -chloroethyl- $\beta$ -hydroxyethylamine (II)]. In a later report (3), Hanby and Rydon stated that the composition of a 1% aged solution was 25% dimer, 20% unchanged MBA, 35% chlorohydrin, and 20% methyldiethanolamine. The values for the last two components were based on analytical rather than isolation data, however.

The fractionation of aged solutions by the picrate method has been repeated in this laboratory. The amounts of dimer and unchanged MBA present in our aged solutions, to judge from the weights and thiosulfate titers of the crude picrate fractions, were about 30% and 10%, respectively. The yield of chlorohydrin was 37% of the MBA employed. Measurements of the thiosulfate titer of the mother liquors, however, indicated that the presence of an additional 7% of chlorohydrin, and about half of this amount was, in fact, isolated as a picrylsulfonate. No methyldiethanolamine could be isolated as a picrate from the mother liquors. The picrate procedure, therefore, permitted the isolation of only about 75% of the original MBA.

It has been found that picrylsulfonic acid (cf. Paper I of this series) is a better reagent than picric acid for the isolation of the components of aged solutions of MBA; cleaner separations and much higher total yields (over 90%) are obtained. The picrylsulfonate isolation procedure was applied to the following 48-hour aged solutions of MBA: (a) a 1% solution of the free base, (b) a 1% solution of

<sup>1</sup> This work was done in whole under Contract No. OEMsr-313 between The Rockefeller Institute for Medical Research and the Office of Scientific Research and Development, which assumes no responsibility for the accuracy of the statements contained herein. The experiments were performed during the period June 1942–January 1944.

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Jied, November 7, 1944.

<sup>4</sup> The structural formulas of this, and the other transformation products of MBA referred to in this paper, are given in Figure 1 in the previous paper of this series. In the present communication, the numbering is unchanged. the base, containing one equivalent of NaCl (from neutralization of the MBA hydrochloride), and (c) a 1.56% (0.10 *M*) solution of the base containing one equivalent of NaCl. The results of these experiments are given in Table I. It will be noted that the presence of NaCl and/or an increase in initial concentration of MBA leads to an increase in the amount of dichloro cyclic dimer and a decrease in the extent of hydrolysis. Under these different conditions, the amount of unchanged MBA remains fairly constant.

In each of these isolation experiments, the results obtained by the picrylsulfonate method were checked against the analytical data obtained on the aged solution before any manipulation had been performed upon it. Such a comparison should reveal changes in the composition of the aged solution produced by the experimental procedures incident to the isolation. The analytical data obtained on the aged solutions just prior to the isolations are given in Table II. The analyses include measurements of the Cl<sup>-</sup> and H<sup>+</sup> liberated, and the 2-hour thiosulfate titer; the difference between the values for the Cl<sup>-</sup> and H<sup>+</sup> represents the Cl<sup>-</sup> neutralized by quaternary nitrogen.

Comparison of the analytical data of the 1% solutions aged in the presence and absence of NaCl shows that the latter has a higher thiosulfate titer but is not significantly different in other respects from the former. It appears that, in the presence of NaCl, the amount of dimer is decreased while the amount of imonium compounds and/or chlorohydrin is increased. The isolation data of Table I show that the solution aged in the absence of NaCl actually has less dimer and more chlorohydrin than is present in the solution aged in the presence of NaCl. Under the conditions of the isolation, any 1-methyl-1-( $\beta$ -hydroxyethyl)ethylenimonium chloride (III) would be expected to revert to the chlorohydrin (II). As was mentioned in the previous paper of this series, a reversion occurs when III picrylsulfonate is allowed to stand in dilute HCl solution.

The 1.56% aged solution of MBA contains more quaternary nitrogen but less H<sup>+</sup> and has a lower thiosulfate titer than does the corresponding 1% solution. This indicates a greater degree of dimerization and less hydrolysis at 1.56% than at 1% concentration.

If no alteration in the composition of the aged solutions had occurred during the isolation procedures, there should be a fairly good quantitative agreement between the analytical values and the corresponding values calculated from the isolation data, since more than 90% of the original MBA has been accounted for in each isolation. Such a comparison is given in Table II. It will be noted that all the carbon-bound chlorine has been accounted for, but that the calculated values for H<sup>+</sup>, Cl<sup>-</sup>, and thiosulfate titer are appreciably lower than the corresponding titration values. This may mean that the material not accounted for is largely methyldiethanolamine and the hydroxyethyl imonium compound III. It is likely that only III and not the chloroethyl imonium compound I would be present because the picrylsulfonate of the latter would have been one of the first to precipitate during the isolation procedure.

The titration data of Table II show that for the aged solutions the thiosulfate titers are higher than are the values for the H<sup>+</sup> liberated. This difference may

be taken as a further indication for the presence of ethylenimonium compounds in the aged solutions. As reported in Paper I of this series, the imonium compound I was isolated from the aged 0.02 M MBA solution. This shows that ethylenimonium compounds may remain in unbuffered solutions of MBA for long periods of time.

From the results given above, it would appear that the composition of 48hour aged solutions of MBA is known within narrow limits. Since the cyclic

concentra- tion of MBA, %	NaCl	COMPONENTS ISOLATED					
		Dichloro cyclic dimer (V), %	Chlorohydrin (II), %	Methyldie- thanolamine (IV), %	Unchanged MBA, %	Total, %	
1 1 1.56	absent present present	22 31 44	58 49 39	2 3 0.3	11 9 8	93 92 91.3	

TABLE I

Composition of 48-Hour Aged Solutions of Methyl-bis( $\beta$ -chloroethyl)amine (MBA)

TABLE II

THE ANALYSIS OF 48-HOUR AGED SOLUTIONS OF METHYL-BIS( $\beta$ -chloroethyl)AMINE (MBA) The values in parentheses are calculated from the isolation data given in Table I.

CONCENTRATION OF MBA %	NACL	CL <sup>-</sup> LIBERATED PER mM MBA M.EQUIV.	CARBON-BOUND CL PER <i>mM</i> MBA M.EQUIV.	H <sup>+</sup> liberated per <i>mM</i> MBA <u>M.EQUIV.</u>	QUATERNARY N(Cl <sup>-</sup> -H <sup>+</sup> ) M.EQUIV.	NA2S2O3 CONSUMED IN 2 HRS. PER MM MBA M.EQUIV.
1	absent	1.00	1.00	0.66	0.34	0.87
		(0.84)	(0.91)	(0.62)	(0.22)	(0.80)
1	present	0.99	1.01	0.64	0.35	0.73
		(0.86)	(0.98)	(0.55)	(0.31)	(0.67)
1.56	present	1.00	1.00	0.54	0.46	0.63
	-	(0.84)	(0.99)	(0.40)	(0.44)	(0.55)

dimer (V) is relatively non-toxic, it is concluded that the toxicity of the aged solutions of MBA is to be attributed to the presence of the chlorohydrin (II), unchanged MBA and possibly also to small amounts of the imonium compound (III).

#### EXPERIMENTAL

Isolation of products from an aged 1% (0.064 M) unbuffered solution of MBA (NaCl absent). One liter of a solution containing 10 g. (64 mM) of MBA (freshly distilled) was maintained at 25° for 48 hours. A 35-cc. aliquot was removed for the analyses reported in Table II. The remainder of the solution was cooled to 0°, acidfied with HCl to Congo Red and then treated with a cold aqueous solution of 22.6 g. (62.0 mM) of picrylsulfonic acid. After standing several hours at 4°, the dipicrylsulfonate of the dichloro cyclic dimer (V) separated; it was filtered off and dried over  $P_2O_5$ ; yield 5.55 g. (22%). The compound did not react with thiosulfate and did not melt up to 250°.

The filtrate was concentrated under reduced pressure to 600 cc. and upon cooling at 4°, the solution deposited the pure chlorohydrin picrylsulfonate; yield 10.0 g. (38%); m.p. and mixed m.p. 141-143°. The compound consumed 0.99 equivalents of thiosulfate in 2 hours.

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The filtrate was concentrated in vacuo to 400 cc. and cooled at 4°. The crude MBA picrylsulfonate (1.4 g.) crystallized (fraction 1); m.p.  $123-128^{\circ}$ ; thiosulfate titer,  $1.75 \ mM$  per mM of MBA picrylsulfonate. The compound was recrystallized twice from 10-cc. portions of hot water containing HCl; yield 0.9 g. (3%) of MBA picrylsulfonate; m.p. and mixed m.p.  $144-146^{\circ}$ ; an authentic sample melted at  $145-147^{\circ}$ . Thiosulfate titer,  $1.96 \ mM$  per mM of MBA picrylsulfonate.

After removal of fraction 1, the filtrate was concentrated to 200 cc. and cooled at  $0^{\circ}$ . A mixture of the MBA and chlorohydrin picrylsulfonates crystallized; yield 1.9 g., (fraction 2); m.p. 125–128°; thiosulfatetiter, 1.3. When recrystallized from acetone, this fraction yielded 0.85 g. (3%) of the chlorohydrin picrylsulfonate; m.p. 141–142°; mixed m.p. 140–142°. The 2-hour thiosulfate titer was 0.97 mM of chlorohydrin salt.

After removal of fraction 2, the filtrate was concentrated to 50 cc. and cooled at 0°; yield 5.9 g. (fraction 3) of a mixture containing mostly the chlorohydrin picrylsulfonate; m.p. 122-130°; thiosulfate titer, 1.1. This fraction was recrystallized from 100 cc. of boiling acetone; yield 3.13 g. of the chlorohydrin picrylsulfonate; m.p. 139-141°; mixed m.p. 139-142°; thiosulfate titer, 0.88. The acetone filtrate was concentrated to 25 cc. and upon cooling deposited a second crop of the chlorohydrin picrylsulfonate; yield 0.9 g. (3%); m.p. and mixed m.p. 139-141°; thiosulfate titer, 0.91. The low thiosulfate titer of the first crop pointed to the presence of about 10% of methyldiethanolamine picrylsulfonate in this fraction. By digesting this material in 100 cc. of boiling acetone and then allowing the mixture to stand at room temperature for 2 hours, crude methyldiethanolamine picrylsulfonate was obtained as an insoluble portion; yield 0.22 g. (0.9 %); m.p. 150-160°. After recrystallization from acetone, it melted at 174-177° and a mixture with the authentic compound melted at 177-180°. The authentic compound melted at 182-183°. From the acetone solution remaining after removal of the crude methyldiethanolamine salt, 2.63 g. of the chlorohydrin picrylsulfonate was recovered by the addition of petroleum ether to the acetone filtrate; m.p. 139-140°; mixed m.p. 139-142°; thiosulfate titer, 0.97 mM per mM of chlorohydrin salt.

After removal of fraction 3, the filtrate was concentrated to 10 cc. and cooled; yield 0.7 g. of a mixture containing methyldiethanolamine, the chlorohydrin (II), and/or MBA picrylsulfonates. It was digested in a small volume of boiling acetone and then allowed to stand several hours at room temperature. The insoluble material (fraction 4) was methyldiethanolamine picrylsulfonate; yield 0.25 g. (1.0%); m.p. 177-180°; mixed m.p. 177-181°.

The mother liquors remaining from working up fractions 1-4 were combined, evaporated nearly to dryness, and the residue was dissolved in 50 cc. of hot water containing HCl. After cooling overnight at 4°, 2.12 g. (8%) of MBA picrylsulfonate crystallized out; m.p. 140-143°; mixed m.p. 142-145°; thiosulfate titer,  $1.92 \ mM$  per mM of MBA picrylsulfonate. The mother liquor was concentrated to 20 cc. and again cooled overnight; yield 1.0 g. (4%) of the chlorohydrin picrylsulfonate; m.p. 136-139°; mixed m.p. 138-141°; thiosulfate titer, 1.02. After recrystallization from acetone the m.p. and mixed m.p. were 141-143° and the thiosulfate titer was 0.98.

The remaining mother liquor was evaporated nearly to dryness. Alcohol was added and the evaporation was repeated. The residue was taken up in 10 cc. of alcohol, cooled at 0°, and filtered; yield 0.45 g. (2%) of crude chlorohydrin picrylsulfonate; m.p. 136-139°; mixed m.p. 138-140°. A small amount (50 mg.) of methyldiethanolamine picrylsulfonate was removed from this portion by digesting it in a little acetone; m.p. 176-179°; mixed m.p. 177-181°. The chlorohydrin salt was recovered from the acetone filtrate by evaporating it and recrystallizing the residue from water; m.p. and mixed m.p. 138-140°; thiosulfate titer, 0.92.

Isolation of compounds from an aged 1% (0.064 M) unbuffered solution of MBA (NaCl present). To 1500 cc. of an aqueous solution containing 18.5 g. (96 mM) of MBA·HCl there was added at 0°, 96 mM of NaOH. The mixture was allowed to stand at room temperature (26°) for 48 hours, then cooled to 0°, acidified with HCl and treated with a cold aqueous

solution of 33.7 g. (96 mM) of sodium picrylsulfonate. After cooling overnight at 4°, the precipitate which had formed was filtered off and washed with cold water and acetone and dried *in vacuo* over P<sub>2</sub>O<sub>5</sub>; yield 12.15 g. (31%) of the dipicrylsulfonate of the dichloro cyclic dimer (V). The compound did not melt up to 250° and was insoluble in and did not react with thiosulfate.

The filtrate was concentrated *in vacuo* to 1400 cc. and cooled overnight at 4°; yield 7.3 g. (18%) of the chlorohydrin picrylsulfonate; m.p. and mixed m.p. 140-142°. The isolated compound consumed 0.99 equivalents of thiosulfate in 2 hours.

After removal of the first fraction of chlorohydrin salt, the filtrate was concentrated to 700 cc. and cooled as before. A mixture of the picrylsulfonates of the chlorohydrin and MBA crystallized out; yield 11.0 g. (fraction 1); m.p. 128-131°; thiosalfate titer, 1.16 mM per mM of chlorohydrin salt.

The filtrate was concentrated *in vacuo* to 300 cc. and upon cooling deposited a mixture of the picrylsulfonates of the chlorohydrin and MBA; yield 3.9 g. (fraction 2); m.p. 127-131°; thiosulfate titer, 1.29. The mother liquor (filtrate A) was reserved for further study.

Fractions 1 and 2 were combined and dissolved in 250 cc. of hot acetone. Upon cooling overnight at 4°, 7.4 g. (18%) of pure chlorohydrin picrylsulfonate crystallized out; m.p. and mixed m.p. 141-143°; thiosulfate titer, 0.94. The mother liquor was concentrated to a volume of 25 cc. Another crop of the chlorohydrin picrylsulfonate crystallized out upon cooling the solution overnight at 4°; yield 1.9 g. (4.6%); m.p. and mixed m.p. 140-142°; thiosulfate titer, 0.98. The acetone mother liquor was evaporated and the residue again dissolved in 25 cc. of warm acetone. Upon standing overnight at room temperature, MBA picrylsulfonate crystallized out; yield 1.8 g. (4.2%); m.p. and mixed m.p. 143-147°; thiosulfate titer,  $1.82 \ mM$  per mM of MBA picrylsulfonate. The acetone residue (2.8 g.) contained the typical crystals of both the chlorohydrin and MBA picrylsulfonates. It was dissolved in 25 cc. of warm acidulated water and cooled for 1 hour at 0°; yield 1.5 g. (3.5%) of MBA picrylsulfonate; m.p. 142-145°; mixed m.p. 143-146°; thiosulfate titer, 1.87 mM per mM of MBA picrylsulfonate. Upon concentration of the filtrate to ca. 10 cc., 0.65 g. ofimpure chlorohydrin picrylsulfonate was obtained (thiosulfate titer, 1.08). It was dissolved in 25 cc. of warm acetone and the solution, upon cooling overnight at 4°, deposited 0.2 g. of the chlorohydrin salt; m.p. and mixed m.p. 140-142°; thiosulfate titer, 0.95.

Filtrate A was evaporated to dryness *in vacuo* and the residue was extracted with 1 liter of boiling acetone. The extract was evaporated and its residue was taken up in 250 cc. of water containing a little HCl. This solution was evaporated to 25 cc., cooled at 4° overnight, and filtered; yield 5.5 g. (fraction 3) of a mixture containing methyldiethanolamine, chlorohydrin and MBA picrylsulfonates. The filtrate was discarded since its thiosulfate titer was negligible (equivalent to 1.3 mM of chlorohydrin picrylsulfonate).

Fraction 3 was dissolved in 200 cc. of boiling acetone. Upon standing for 3 hours at room temperature, the solution deposited 0.7 g. (1.8%) of methyldiethanolamine picrylsulfonate. This compound did not react with thiosulfate; m.p. 178–180°; mixed m.p. 179–182°.

The mother liquor was concentrated to 50 cc. in two stages to yield 2.2 g. of chlorohydrin picrylsulfonate mixed with a little methyldiethanolamine picrylsulfonate; m.p. 139-142°; mixed m.p. 139-143°. The thiosulfate titer of the impure salt was 0.81, indicating that it contained about 15% of methyldiethanolamine.

The acetone residue, 1.1 g., was not fractionated further. From its thiosulfate titer of 1.4 and its chlorine content of 12.15% it is estimated to be a mixture of picrylsulfonates containing about 45% MBA picrylsulfonate and about 55% chlorohydrin picrylsulfonate. (A 10-15% error in this estimation will make no apparent difference in the over-all yields.)

Isolation of products from a 1.56% (0.1 M) aged unbuffered solution of MBA (NaCl present). To 500 cc. of an aqueous solution containing 9.93 g. (51.5 mM) of MBA·HCl was added 51.5 mM of NaOH. The mixture was maintained at 25° for 48 hours. After removal of a 25 cc. aliquot for titration, the remainder of the solution was cooled to 0°, acidified to Congo Red with HCl and treated with a cold solution of 17.4 g. (49.5 mM) of sodium picrylsulfonate dissolved in 210 cc. of 0.35 N HCl. After cooling overnight at 4°, the precipitate of the dipicrylsulfonate of the dichloro cyclic dimer (V) was filtered off and dried; yield 9.05 g. (44%). The compound showed the same properties as in the previous experiment.

The filtrate was concentrated *in vacuo* to 125 cc. and then cooled for 2 days at 4°. Crude chlorohydrin picrylsulfonate (9.05 g., fraction 1) was thus obtained; m.p. 131-135°; thiosulfate titer,  $1.13 \ mM$  per mM of chlorohydrin salt.

The filtrate was evaporated to dryness *in vacuo* and the residue was extracted with 1 liter of boiling acetone. After evaporation of the acetone, the residue was taken up in 25 cc. of warm water containing a little HCl and then cooled; yield 1.8 g. (fraction 2) of crude chlorohydrin picrylsulfonate; m.p. 128-133°; thiosulfate titer, 0.95 mM per mM of chlorohydrin picrylsulfonate. The mother liquor (filtrate A) was reserved for further treatment.

Fractions 1 and 2 were combined and recrystallized from 200 cc. of warm acetone; yield 6.2 g. (29%) of pure chlorohydrin picrylsulfonate; m.p. and mixed m.p. 141-144°. The compound consumed 0.94 equivalents of thiosulfate in 1 hour.

The mother liquor was evaporated and the residue was dissolved in 25 cc. of warm acetone. After cooling overnight at 4°, another crop of the chlorohydrin salt was obtained; yield 1.4 g. (7%); m.p. 139-141°; mixed m.p. 140-143°; thiosulfate titer, 0.94. Evaporation of the mother liquor left 2.35 g. of a mixture composed mainly of chlorohydrin and MBA picrylsulfonates (thiosulfate titer, 1.7). This residue was dissolved in 20 cc. of warm acidulated water and then cooled for 1 hour at 0°; yield 1.8 g. (8%) of MBA picrylsulfonate. The compound consumed 1.89 equivalents of thiosulfate in 2 hours; m.p. 139-143°; mixed m.p. 140-144°. Crystals of the chlorohydrin picrylsulfonate appeared in the filtrate upon standing at 4°; yield 0.2 g. (1%); m.p. 131-134°; mixed m.p. 134-139°. Thiosulfate titer, 0.97 mM per mM of chlorohydrin salt. After recrystallization from acetone-petroleum ether, its m.p. was 140-142°; mixed m.p. 141-143°.

Filtrate A was concentrated to about 5 cc. and upon cooling deposited 0.6 g. of chlorohydrin picrylsulfonate mixed with a little methyldiethanolamine picrylsulfonate; m.p. 125-130°; mixed m.p. 130-133°; thiosulfate titer 0.85. When dissolved in acetone and allowed to stand at room temperature, the picrylsulfonate of methyldiethanolamine crystallized out; yield 70 mg. (0.3%); m.p. 179-181°; mixed m.p. 179-182°. The compound did not react with thiosulfate. The chlorohydrin picrylsulfonate crystallized from the mother liquor upon addition of petroleum ether; yield 0.35 g. (2%); m.p. 139-142°; mixed m.p. 139-143°.

Isolation of products from a 1% (0.064 M) aged unbuffered solution of MBA by the picrate method (NaCl present). A 1% solution (1500 cc.) of MBA was prepared and aged as before. After 2 days, it was treated with 195 cc. of 0.5 N calcium picrate solution. After 1 hour at room temperature, the precipitate which had formed was filtered and dried; yield 10.0 g. of crude dipicrate of the dichloro cyclic dimer (V); m.p. 164-176°. The compound was only slightly soluble in, and did not react with, aqueous thiosulfate. After recrystallization from water, the compound melted at 183-187° and a mixture with an authentic sample melted at 189-193°. The pure compound melted at 201-203°.

The filtrate was concentrated *in vacuo* to 1 liter (bath temperature 40°) and cooled overnight at 4°. The fraction which separated was evidently a mixture of the picrates of the dichloro cyclic dimer and MBA since it was not completely soluble in thiosulfate and contained no detectable amounts of the large orange prisms of the chlorohydrin salt; yield 5.6 g.; m.p. 114-120°; thiosulfate titer, 1.30. From its thiosulfate consumption, it is estimated to contain about 70% MBA picrate (10%). After recrystallization from water, its m.p. was 120-124°; mixed m.p. 120-128°. The pure compound melted at 129-131° and consumed 1.94 equivalents of thiosulfate in 2 hours.

The filtrate was concentrated *in vacuo* to 600 cc. and, upon cooling overnight at 4°, deposited the large orange prisms of the chlorohydrin picrate; yield 11.0 g. (31%); m.p. 66-68°; thiosulfate titer, 1.00 mM per mM of chlorohydrin picrate. A second crop of the chlorohydrin was obtained by concentrating the mother liquor to 300 cc. and cooling as before; yield 2.1 g. (6%); m.p. 66-69°; thiosulfate titer, 0.99. The two crops were combined and

recrystallized from ethyl acetate-benzene to yield 11.2 g. of the pure chlorohydrin picrate; m.p. 70-73°.

Anal. Calc'd for  $C_5H_{12}CINO \cdot C_6H_3N_3O_7$ : C, 36.0; H, 4.1.

Found: C, 35.7; H, 4.25.

Attempts to isolate any further compounds from the aqueous filtrate yielded only calcium picrate. However, the thiosulfate titer of an aliquot of the filtrate indicated the presence of  $6.4 \ mM$  (6.7%) of chlorohydrin in the mother liquor. Part of this was isolated by adding an aqueous solution of sodium picrylsulfonate to the aqueous mother liquor, discarding the initial precipitate and allowing the filtrate to stand at room temperature. Crystals of the chlorohydrin picrylsulfonate soon appeared; yield 1.3 g.; m.p. 139-142°; mixed m.p. 139-143°; thiosulfate titer, 0.94 mM per mM of chlorohydrin salt.

The authors wish to acknowledge with thanks the helpful cooperation of Miss Rosalind E. Joseph, who assisted in the conduct of these experiments, and of Mr. Stephen M. Nagy, who performed the microanalyses reported in this paper.

NEW YORK, N. Y.

### REFERENCES

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(2) HANBY AND RYDON (1942).<sup>a</sup>

(3) BOYLAND (1942).ª

<sup>a</sup> Unpublished data obtained in Great Britain.