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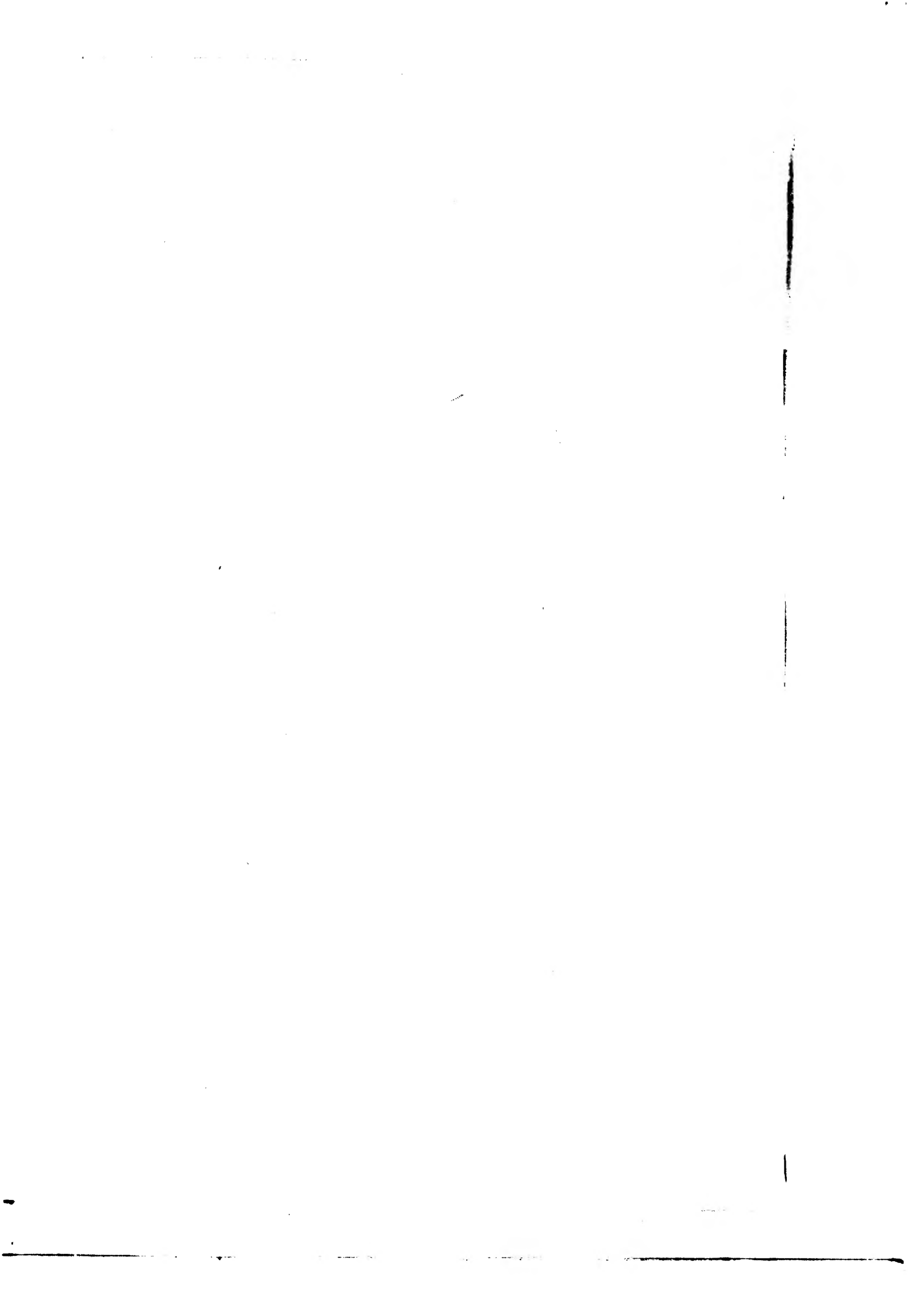
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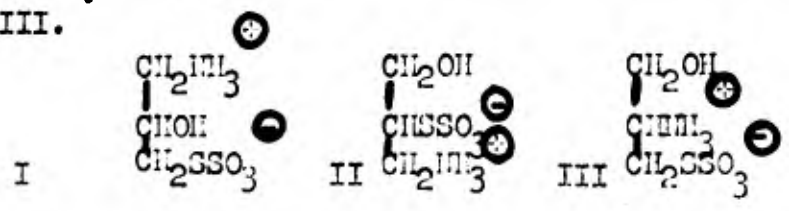
POTENTIAL RADIATION-PROTECTIVE COMPOUNDS.
SYNTHESIS OF THE THREE ISOMERIC THREE-CARBON
AMINOHYDROXY BUNTE SALTS AND RELATED COMPOUNDS ¹

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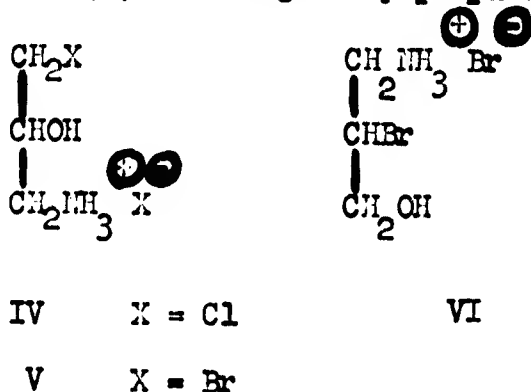
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In recent years, Bunte salts, S-alkyl thiosulfates, have been the subject of investigation in many laboratories. A Bunte salt related to glutathione, "S-sulfoglutathione", has been isolated from calf lens extracts.⁴ Sulfite has been used to obtain soluble protein or peptide fractions from wool,⁵ flour,⁶ ribonuclease and insulin,⁷ and from trypsinogen and alpha-chymotrypsinogen;⁸ the cystine disulfide bonds are cleaved under mild conditions to form "S-sulfocysteinyl residues". The properties and potential uses of the "S-sulfocysteinyl residues" have been discussed by Swan.⁹

Several examples of Bunte salts containing amino or alkyl-amino groups were prepared by Bretschneider.¹⁰ These, like the amino-acids, are internal salts and the simplest compound of this type, S-2-aminoethyl thiosulfuric acid, was found to have significant radiation-protective activity in mice. It was also 2.4 times less toxic than 2-aminoethanethiol (cysteamine) hydrochloride.¹¹ Other aminoalkyl thiosulfates have been prepared by Rosenthal and Citarel¹² who found that they were stable compounds which possessed significant anti-radiation activity. The low activity of 3-amino-1-propanethiol compared to the activity of S-3-aminopropyl thiosulfuric acid¹³ suggests that the protective activity of the Bunte salt is not, at least in this case, due to the formation of the thiol.

Aminoalkyl thiosulfuric acids are, in general, stable, odorless, crystalline, water-soluble substances and are thus attractive potential anti-radiation drugs. This paper describes the synthesis of the three isomeric internal Bunte salts I, II and III.



Bunte salts are conveniently prepared by the reaction of alkyl halides with thallos thiosulfate,¹⁴ the insoluble thallos halide formed being easily removed from the reaction mixture. An aqueous solution of 1-amino-3-chloro-2-propanol hydrochloride (IV),^{15,16} unlike 2-chloroethylamine hydrochloride,¹⁴ did not react readily with thallos thiosulfate at room temperature. Formation of the internal Bunte salt took place at higher temperatures but extensive decomposition also occurred. The corresponding bromo hydrobromide (V) was originally prepared by condensation of



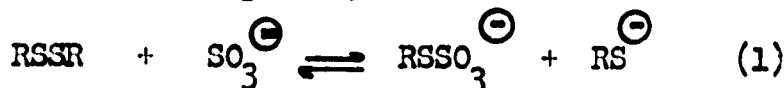
potassium phthalimide with boiling epibromohydrin, followed by hydrolysis of the product with hydrobromic acid.¹⁷ When *N,N*-dimethylformamide was added in the condensation step,¹⁸ the reaction proceeded at 35-40° and the yield was improved slightly. Under these conditions, the epoxide ring is slowly opened by the potassium phthalimide. This was demonstrated by the formation of a small amount of a diamino-propanol (presumably 1,3-diamino-2-propanol) after hydrolysis of the condensation product. The bromo hydrobromide reacted more readily than IV with thallos thiosulfate and, after 2 days at 50°, the Bunte salt I, *S*-3-amino-2-hydroxypropyl thiosulfuric acid, was isolated in 67% yield.

No suitable precursor for the Bunte salt II was known and attempts to prepare 3-amino-2-bromo-1-propanol hydrobromide (VI) from 2,3-dibromo-1-propanol and from 2,3-dibromopropylamine and their derivatives, by selective displacements of the primary bromine atoms, were unsuccessful. The hydrobromide (VI) was eventually obtained from the reaction of the allylammonium ion with hypobromous acid. The latter was conveniently generated *in situ* by the method of Leibman and Fellner¹⁹ whereby a mixture of bromine vapor and air is passed into the reaction solution which contains one equivalent of silver nitrate. Both isomeric aminobromo alcohols were isolated, the desired 3-amino-2-bromo isomer VI being the major product. The hydrobromide VI did not react with thallos thiosulfate at 40° but, at 75-80°, conversion to the Bunte salt II was essentially complete after 12 hours. Amine impurities, which hindered crystallization, were removed by chromatography on silica gel. Crystallization of the purified material from methanol-ether gave the pure Bunte salt II, *S*-2-amino-1-(hydroxymethyl)ethyl

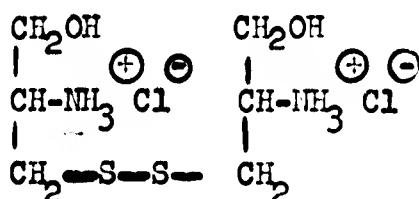
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thiosulfuric acid in 51% yield.

Bunte salts have also been prepared by the action of sulfites on disulfides in the presence of an oxidizing agent. Air or oxygen are often sufficient but cupric ions,²⁰ iodosobenzoate and tetrathionate⁷ have also been used. The oxidant converts the thiol formed in the reversible reaction (1) to disulfide and eventually this is completely converted to the Bunte salt.



Cystinol dihydrochloride (VII), 3,3'-dithiobis [2-amino-1-propanol] dihydrochloride, is therefore a suitable precursor for the internal Bunte salt III. It was prepared from 2-phenyl-2-thiazoline-4-methanol by Crawhall et al.²¹ These authors designated their product as the DL-form but the method of preparation would give

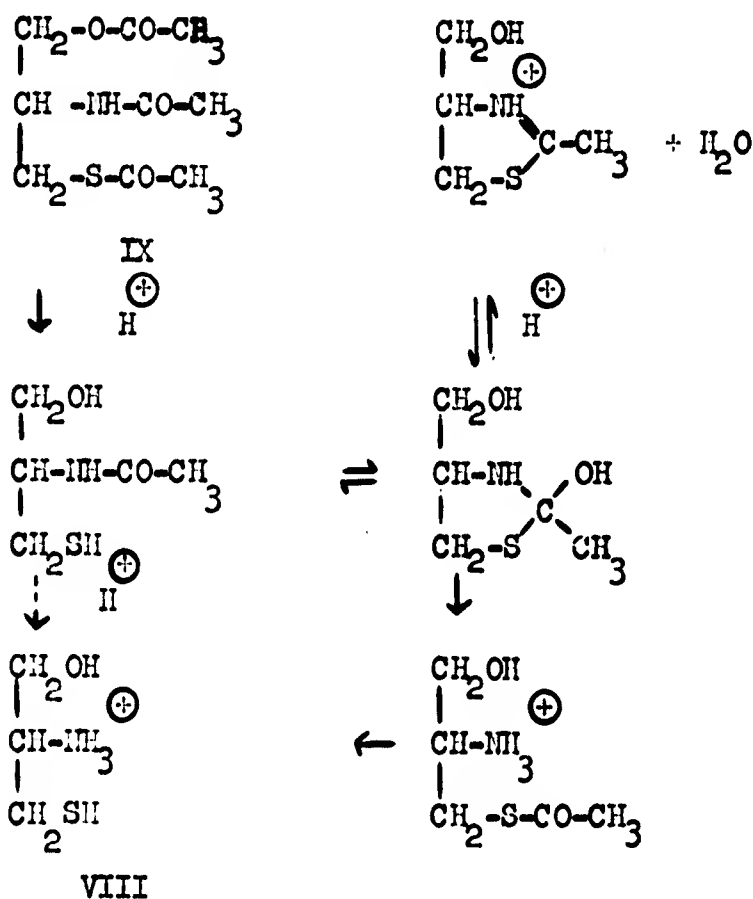


VII

a mixture of DL- and meso-isomers. We used the L-isomer of the thiazoline in an attempt to obtain the pure L-isomer of VII but hydrolysis of the thiazoline ring required much more vigorous conditions than those reported²¹ and racemization occurred at this stage. The resultant DL-thiol ("cysteinol") was oxidized without isolation and a crystalline, optically inactive mixture of, presumably, the DL- and meso- forms of VII was obtained. No attempt was made to separate these isomers and the mixture was treated at room temperature with ammonium sulfite solution (pH 7) and oxygen. The reaction was followed by paper electrophoresis and was complete after 6-7 hours. The product was freed from inorganic salts by fractionation on a column of a cation exchange resin in the lithium salt form²² and subsequent recrystallization from methanol-ether gave S-2-amino-3-hydroxypropyl thiosulfuric acid (III) in 65% yield.

L-Cysteinol, L-2-amino-3-mercapto-1-propanol (VIII), has recently been prepared by Enz and Cecchinato²³ by a method which should not cause appreciable racemization. L-Cysteine ethyl ester was reduced with lithium aluminum hydride and the L-cysteinol formed was isolated as the N,O,S-triacetate (IX) in 29% yield. These authors hydrolyzed the triacetate with dilute hydrochloric acid and obtained L-cysteinol as the crystalline hydrochloride, although they

did not record the specific rotation. Thin layer chromatography indicated that during hydrolysis of the triacetate, an intermediate was rapidly formed and that this was slowly converted to cysteinol. In dilute hydrochloric acid at 90°, the absorption at 231.5 mμ (thiolacetate) decreased rapidly. A second absorption at 261 mμ (thiazolinium cation) appeared, increased to a maximum after ca 1½ hours and then decreased slowly (Fig.1). It has been shown recently that thiazoline formation occurs in acid solutions of N-acetyl cysteine²⁴ and N-2-mercaptoethyl-acetamide²⁵ and hydrolysis of cysteinol triacetate (IX) probably proceeds via the same mechanism.



L-Cystinol dihydrochloride was prepared from L-cysteinol triacetate by acid hydrolysis followed by oxidation of the resultant thiol which was not isolated. The L-form of VII had a specific rotation of -108° and a much lower melting point than the values obtained for the mixtures of DL- and meso- forms.

The internal Bunte salt (III) prepared from this disulfide had a higher melting-point than the DL-form and a specific rotation of -31° in water.

The three isomeric internal Bunte salts are stable crystalline solids, very soluble in water and slightly soluble in methanol. They decompose slowly in boiling water. All three compounds showed slight activity when tested as radiation-protective

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Footnotes

- (1) This work was supported by a grant from the Surgeon General's Office, Medical Research and Development Command, U.S. Army.
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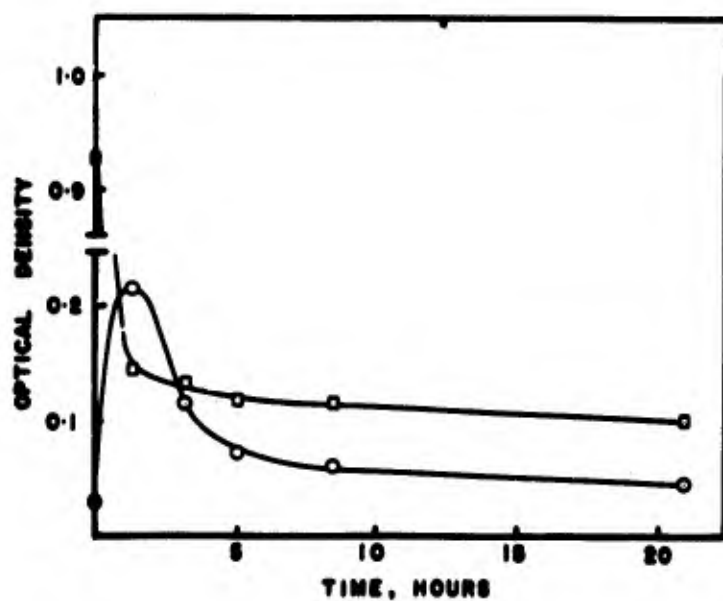


Fig. 1. -- Hydrolysis of L-cysteinol triacetate (2.08×10^{-4} M) in hydrochloric acid (1.7 N) at 90° . \square - optical density at 231.5 mμ. \circ - optical density at 261 mμ.