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THE STRUCTURE OF THE MUSTARD OIL GLUCOSIDES

AND

SYNTHESIS OF THE GLUCOTROPAEOLATE ION

by

Allan Jay Lundeen

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The author wishes to express his sincere appreciation to Dr. M. G. Ettlinger, who conceived this problem and expertly directed its solution.

Also the author wishes to express his thanks to the National Science Foundation for financial support.
To my wife Janet
and my Parents
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Section I
Introduction

Since the first isolation of allyl isothiocyanate from the seeds of black mustard over a century ago, the occurrence of isothiocyanates in nature has frequently been reported. Organic isothiocyanates are commonly called mustard oils because of their long recognized distribution in plants of the mustard family, Cruciferae, which derived the name originally from the French condiment moutarde. Various of the mustards have been important items of commerce since ancient times.

Mustard oils may be conveniently grouped as volatile and non-volatile. Volatile oils, such as allyl mustard oil, have a characteristically sharp taste and odor, whereas the non-volatile, such as p-hydroxybenzyl isothiocyanate (the active principle of table mustard), are odorless but also possess a sharp taste. Volatile mustard oils may be easily separated from the seed mixture by steam distillation, and consequently have been more extensively investigated than non-volatile mustard oils.

Mustard oils do not occur free in plants but are formed from glucosides on maceration by the action of water and an enzyme which occurs in the plants. At the present time, about two dozen mustard oil glucosides have been detected and at least six have been isolated in crystalline condition. Interest in these compounds is stimulated by the relation of one of these glucosides to an antithyroid substance, and by their extensive distribution in plants used for human and animal consumption. The purpose of this investigation is to elucidate the chemical nature of the precursors of mustard oils in plants, the mustard oil glucosides.
Previous Investigations of Mustard Oil Glucosides

Botron and Robiquet 3 and also Faure 4 recognized that allyl mustard oil did not occur free in the seeds of black mustard but was liberated by the addition of water to the crushed seeds. They found that seeds warmed in alcohol or treated with other reagents that would coagulate proteins did not liberate allyl mustard oil by the addition of water. Botron and Freme 5 isolated a protein fraction from aqueous extracts of black or white mustard seed, which in aqueous solution would liberate allyl mustard oil from alcohol-treated black mustard seeds. Similar observations were made by Bussy, 6 who succeeded in isolating a crystalline glucoside from the seeds of black mustard as a potassium salt, which he called potassium myronate. The enzyme present in black and white mustard, which Bussy demonstrated would liberate allyl mustard oil from the glucoside, he called myrosin. Bussy detected sulfate as a decomposition product of potassium myronate but made no further attempt to elucidate the structure of this glucoside which is today called sinigrin. These workers recognized the similarity between the hydrolysis of sinigrin by myrosin and the cleavage of amygdalin by emulsin. 7

Sinalbin, the first mustard oil glucoside isolated, was obtained from white mustard in 1831. 8 Because of its complexity, it was not the object of as extensive chemical investigation as sinigrin.

Bussy's work was disputed for a time but was confirmed by Ludwig and Lange 9 and by Will and Korner 10 as well as by subsequent workers.
Will and Korner found the empirical formula of sinigrin to be $C_{10}H_{18}O_{10}N^-S_2K$ and investigated a number of its reactions. Although enzymatic degradation produced principally allyl mustard oil, glucose and potassium bisulfate, small amounts of sulfur and presumably allyl cyanide were formed. Hydrolysis with water under severe conditions ($110\degree-120\degree$ in a sealed tube) furnished hydrogen sulfide and allyl cyanide but no mustard oil.* Barium hydroxide but not barium chloride reacted rapidly with sinigrin in the cold producing barium sulfate. The barium sulfate thus obtained accounted for one half of the sulfur content of sinigrin. A small amount of barium hydroxide warmed with a large excess of sinigrin produced some mustard oil, but when an excess of barium hydroxide was used, no mustard oil was detected.

Of special interest was the reaction of sinigrin and aqueous silver nitrate to liberate glucose and form an insoluble white precipitate called silver sinigrate. This rather unstable material had the composition of silver sulfate plus allyl mustard oil. Silver sinigrate liberated allyl mustard oil by the action of heat, metal chlorides, sulfides or thiosulfates, whereas acidic reagents such as hydrogen sulfide quantitatively produced allyl cyanide and free sulfur.

The first structural proposal for sinigrin was made by Gadamer¹¹ largely on the basis of evidence furnished by Will and Korner. Gadamer confirmed the observation of Birkemwald¹² that sinigrin was a monohydrate and the molecular formula was correctly represented as $C_{10}H_{16}O_{10}N^-S_2K \cdot H_2O$. The rapid enzymatic cleavage of sinigrin to

* A small amount of acid would be present due to the generation of potassium bisulfate by the decomposition of sinigrin.
furnish allyl isothiocyanate under mild conditions was interpreted by Gadamer to mean that this structural unit was present in the mustard oil glucoside. The cleavage of sinigrin by silver nitrate with liberation of glucose indicated that the sulfate group was not attached to the glucose residue. In view of the tenacity with which silver ion combines with sulfur to form mercaptides, Gadamer believed that silver ion displaced glucose from the sulfide sulfur atom of sinigrin, that is, the sulfur not contained in the sulfate group. Hence Gadamer proposed that sinigrin was represented by the formula I and silver sinigrate by formula II. The reactions of sinigrin known to Gadamer are listed in Table I.

\[
\begin{align*}
I & : R-N=C-S-(C_6H_{11}O_5) \quad & R-N=C-S-Ag \quad & \text{II} \\
& \quad \quad \quad \quad \quad OSO_3K^+ & \quad \quad \quad \quad \quad OSO_3Ag^+ \\
R & = CH_2=CH-CH_2- 
\end{align*}
\]

Sinalbin and all other mustard oil glucosides have been assumed by analogy to be of the same type with \((R)\) corresponding to the particular mustard oil. All mustard oil glucosides investigated show the characteristic reactions with silver nitrate and with myrosin. While there are probably several similar enzymes which collectively are called myrosin, it is worthy of note that all mustard oil glucosides, insofar as has been determined, may be hydrolyzed by the same myrosin preparation. The myrosin enzymes appear to hydrolyze only this class of glucosides. The similarity of the reactions of different mustard oil glucosides is further emphasized by the consideration of specific examples.
Table (I)

Reactions of Sinigrin Known to Gadamar

Sinigrin (C_{10}H_{16}O_{9}S_{2}K + H_{2}O)

- Myrosin + H_{2}O → CH_{2}=CH−CH_{2}NCS + KHSO_{4} + C_{6}H_{12}O_{6}
  (Trace of S and CH_{2}=CH−CH_{2}=CN)
- H_{2}O, H^{+}, Heat → CH_{2}=CH−CH_{2}CN + H_{2}S + KHSO_{4} + C_{6}H_{12}O_{6}
- AgNO_{3} → C_{4}H_{5}O_{4}HS_{2}Ag_{2} (Silver sinigrate) + C_{6}H_{12}O_{6}
- BaCl_{2} → CH_{3}CH=CH−COOH + BaSO_{4} + C_{6}H_{12}O_{6}
- Small amount Ba(OH)_{2} → CH_{2}=CH−CH_{2}NCS + BaSO_{4}

Silver Sinigrate (C_{4}H_{5}O_{4}NS_{2}Ag_{2})

- Cl⁻ etc. or heat → CH_{2}=CH−CH_{2}NCS + 2AgCl + H_{2}SO_{4}
- H_{2}S or HCl etc. → CH_{2}=CH−CH_{2}CN + H_{2}SO_{4} + S + Ag_{2}S
The glucoside of benzyl mustard oil, glucotropaeolin, had not been obtained pure prior to this work. The synthesis of the glucotropaeolate ion is described in the following section. It was detected by Gadamer in *Tropaeolum majus* and *Lepidium sativum* and shown to be hydrolyzed by myrosin to benzyl isothiocyanate, glucose and potassium bisulfate. By the action of silver nitrate on partly purified solutions of the glucoside, Gadamer obtained silver glucotropaeolate which afforded benzyl isothiocyanate by the action of sodium thiosulfate and benzyl cyanide with hydrochloric acid. Heating solutions of the glucoside in water or dilute acid produced benzyl cyanide and hydrolysis in stronger acid furnished phenylacetic acid.

Sinalbin, described previously, was hydrolyzed to p-hydroxybenzyl mustard oil, sulfate and glucose by myrosin but furnished p-hydroxyphenylacetic acid by prolonged heating with a solution of barium chloride. Sinalbin yielded a silver derivative on reaction with silver nitrate, which was decomposed to p-hydroxyphenylacetonitrile with hydrogen sulfide.

Glucocheirolin, the glucoside of the mustard oil cheirolin (3-methylsulfonylpropyl isothiocyanate) was isolated in crystalline form from the seeds of *Cheiranthus cheiri* and was the third mustard oil glucoside to be isolated. It reacted with silver nitrate in a manner analogous to that of sinigrin to yield a silver derivative. Silver cheirolate reacted with chloride ion to furnish the mustard oil.

Similarly, the glucoside of sulforaphen (4-methylsulfoxy-3-butenyl isothiocyanate) yielded a silver salt with silver nitrate.
silver salt furnished the mustard oil and a small amount of nitrile when treated with sodium thiosulfate.

In summary, mustard oil glucosides react under suitable conditions to produce either mustard oil or a nitrile containing the same number of carbon atoms and with the next higher carbon chain. In view of these reactions and particularly the uniqueness of the reaction with myrosin, the conclusion that all mustard oil glucosides are chemically similar is inescapable.

In spite of the fact that the Gadamer structure for mustard oil glucosides provides no ready explanation for nitrile formation, it has remained without revision for nearly sixty years. Subsequent detection of thioglucone as di-β-glucosyl disulfide octaacetate in small yield by the action of aqueous sodium hydroxide on sinigrin has been interpreted as support for the Gadamer structure.¹⁹

In a synthetic approach to the mustard oil glucoside problem, Schneider et al. prepared a series of thiourethane glucosides (III) from silver salts of thiourenes and acetobromoglucose.²⁰ These compounds, modeled after the Gadamer structure I, showed no particular resemblance to mustard oil glucosides in chemical behavior.

Schneider obtained crystalline thiourethane glucoside tetraacetates, which from their mode of preparation must have been β-thioglucopyranosides. The free glucosides, not obtained in crystalline form, were not cleaved by myrosin.

\[
\begin{align*}
\text{R} & \quad \text{N} = \text{C} \\
\text{S} & \quad \text{C}_6\text{H}_{11}\text{O}_5 \\
\text{C} & \quad \text{C}_2\text{H}_5 \\
\text{III} & \quad 
\end{align*}
\]
It is to interest to note that the optical rotations of mustard oil glucosides are similar to those of Schneider's glucoside acetates and other \( \beta \)-l-thioglucopyranosides. Several examples are compared in Table II. This implies that mustard oil glucosides are \( \beta \)-thioglucosides, but leaves the question of ring size unsettled. Schneider et al. found \( \alpha \)-glucose to be liberated initially by the action of silver nitrate on sinigrin and sinalbin.\(^{19}\) \( \alpha \)-Glucose was also the initial product when silver nitrate reacted with sodium \( \beta \)-thiogluco-losate, in support of the contention that mustard oil glucosides are \( \beta \)-thio-gluco-sides.

In recent years, work with mustard oil glucosides has been of a preparative and survey nature. Paper chromatography has been proven a useful tool in the detection and identification of mustard oil glucosides,\(^{22}\) and ion exchange chromatography has aided in their isolation.\(^{23}\) In view of the increasing prominence of mustard oil glucosides, evidence concerning their chemical nature requires re-evaluation in light of modern concepts.
<table>
<thead>
<tr>
<th>Compound</th>
<th>$\frac{M}{D}$</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinigrin tetraacetate*</td>
<td>$-105^\circ$</td>
<td>Water</td>
</tr>
<tr>
<td>Sinigrin*</td>
<td>$-72^\circ$</td>
<td>Water</td>
</tr>
<tr>
<td>Tetramethylammonium tetraacetylglucotropaeolate*</td>
<td>$-123^\circ$</td>
<td>Water</td>
</tr>
<tr>
<td>Tetramethylammonium glucotropaeolate*</td>
<td>$-81^\circ$</td>
<td>Water</td>
</tr>
<tr>
<td>0-Ethyl $S$- $\beta$-d-1-(tetraacetyltioglycopyranosyl)-allyliminothiocarbonate\textsuperscript{20}</td>
<td>$-85^\circ$</td>
<td>Acetylene tetrachloride</td>
</tr>
<tr>
<td>0-Ethyl $S$- $\beta$-d-1-(tetraacetyltioglycopyranosyl)-benzyliminothiocarbonate\textsuperscript{20}</td>
<td>$-18^\circ$</td>
<td>Acetylene tetrachloride</td>
</tr>
<tr>
<td>Ethyl tetraacetyl-$\chi$-d-1-thioglycopyranoside\textsuperscript{21a}</td>
<td>$819^\circ$</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Ethyl tetraacetyl-$\beta$-d-1-thioglycopyranoside\textsuperscript{21b}</td>
<td>$-108^\circ$</td>
<td>Acetylene tetrachloride</td>
</tr>
<tr>
<td>Ethyl $\chi$-d-1-thioglycopyranoside\textsuperscript{21a}</td>
<td>$605^\circ$</td>
<td>Water</td>
</tr>
<tr>
<td>Ethyl $\beta$-d-1-thioglycopyranoside\textsuperscript{21b}</td>
<td>$-135^\circ$</td>
<td>Water</td>
</tr>
</tbody>
</table>

\textsuperscript{*}Experimental, Section II or III.
Section II
The Structure of Sinigrin and Sinalbin

Sinigrin and sinalbin, the two longest known and most accessible mustard oil glucosides, were chosen for structural studies. Sinigrin was readily obtained from extracts of black and brown mustard seeds with aqueous acetone by the use of ion exchange chromatography, a procedure much less tedious than previously used. Sinigrin could be converted into crystalline cesium, rubidium, ammonium, tetramethylammonium and thallous salts by ion exchange resins. The sodium and lithium salts, prepared by the same method, could not be crystallized. In general, it appeared that the salts of the larger cations were easier to obtain crystalline. This observation was utilized in the isolation of glucotropaolin (described in section III) which has repeatedly failed to crystallize as the potassium salt but which crystallizes very readily as the tetramethylammonium salt. This technique would possibly be useful in the isolation of other mustard oil glucosides which do not crystallize easily as potassium salts.

Sinalbin crystallizes directly from aqueous-alcoholic extracts of white mustard seeds as the sinapine (IV) salt.

\[ \text{CH}=\text{CH}-\text{COCH}_2\text{CH}_2-\text{N(CH}_3\text{)}_3 \]

\[ \text{IV} \]
As the sinapine ion would interfere in the chemical investigation of the glucosinalbate residue, sinalbin was converted into the crystalline tetramethylammonium salt by cation exchange. Although tetramethylammonium glucosinalbate could be prepared directly by passing a sinalbin solution through Amberlite IR-120 cation exchange resin in tetramethylammonium form, it was advantageous to first precipitate most of the sinapine \textsuperscript{13} by addition of potassium thiocyanate because of the low capacity of the resin for sinapine.\textsuperscript{25}

Chemical investigation of sinigrin and tetramethylammonium glucosinalbate was initiated by the study of Raney nickel reduction of these glucosides. Organic sulfur compounds have previously been found to undergo reductive desulfurization with an excess of Raney nickel\textsuperscript{26} and this reaction has been widely applied in carbohydrate chemistry.\textsuperscript{27} Raney nickel preparations have been used to reduce a variety of functional groups, usually under mild conditions.\textsuperscript{28}

Sinigrin underwent reductive cleavage and hydrogenation of the olefinic bond to furnish \textit{n}-butylamine, isolated as the \textit{p}-nitrobenzamide, hydrochloride and \textit{m}-nitrobenzenesulfonamide. The hydrolysis was slightly exothermic, proceeding rapidly in aqueous solution at room temperature. Tetramethylammonium glucosinalbate reacted with Raney nickel under the same conditions to give 2-(\textit{p}-hydroxyphenyl)-ethylamine (tyramine), isolated as the hydrochloride and further identified as the dibenzoate.

Hydrolysis of sinigrin in 3 M sulfuric acid furnished vinyl-acetic acid, identified as the \textit{p}-bromophenacyl and \textit{p}-phenylphenacyl esters. Tetramethylammonium glucosinalbate on hydrolysis with
3 M sulfuric acid yielded p-hydroxyphenylacetic acid. Both sinigrin and tetramethylammonium glucosinalbate under these conditions furnished hydroxylamine, identified by paper chromatography and isolation as fluorenone oxime. The maximum yield of hydroxylamine was realized with 3-4 M sulfuric acid (59-62%) while hydrolysis under the same conditions by 1 M sulfuric acid produced only a 25% yield of hydroxylamine. Hydrolysis of mustard oil glucosides by water or dilute acid apparently leads mainly to nitrile formation.\textsuperscript{10,11,14,15,16} In one experiment,\textsuperscript{16} glucotropaeolin was hydrolyzed by 15% hydrochloric acid to furnish phenylacetic acid. Hydroxylamine was undoubtedly formed to some extent under these conditions but it was not detected.

To obtain additional evidence about the nature of the glucoside portion of mustard oil glucosides, sinigrin tetraacetate and tetramethylammonium pentaacetylglucosinalbate were subjected to Raney nickel hydrogenolysis. The known sinigrin tetraacetate\textsuperscript{30} by Raney nickel hydrogenolysis yielded polygalitol tetraacetate which could be deacetylated to polygalitol (V). Tetramethylammonium glucosinalbate was converted to the previously unknown pentaacetyl derivative which also furnished polygalitol tetraacetate by Raney nickel hydrogenolysis. Since polygalitol has been shown to have a six-membered ring, mustard oil glucosides must be 1-thioglucopyranosides.

![Diagram of polygalitol (V)](image-url)
This is in agreement with the observation that l-thioglucose is produced by the action of sodium hydroxide on sinigrin.\(^{19}\) Consideration of the optical rotations of sinigrin, sinalbin and other mustard oil glucosides, together with the evidence of Schneider,\(^{19}\) leads to the conclusion that mustard oil glucosides are \(\beta\)-l-thioglycopyranosides.

The fragments produced by the chemical degradation of mustard oil glucosides can be combined only in the following general formula.

\[
R-(C,N)_{\text{OSO}_3} S-(C_6H_{11}O_5)
\]

The Gadamer proposal (I) and formulas VI and VII are the only possible expansions of this general formula.

\[
\begin{align*}
S-(C_6H_{11}O_5) & \quad S-(C_6H_{11}O_5) & \quad \text{OSO}_3 \\
R-N=N=OSO_3 & \quad R-C=N=OSO_3 & \quad R-C=N-S-(C_6H_{11}O_5)
\end{align*}
\]

I \quad VI \quad VII

Although the Gadamer formula (I) offers a rationale for the formation of mustard oil (RNGS), thioglucose, and polygalitol, the derivations of a primary amine (RCH\(_2\)NH\(_2\)), a carboxylic acid (RCOOH) or hydroxylamine on the basis of this structure are mechanically unlikely. Structure VII suggests no route to mustard oil and would presumably hydrolyze in acid like known sulfenamides\(^{32}\) to give ammonia. Both structures VI and VII provide a basis for the formation of polygalitol, thioglucose, a primary amine (RCH\(_2\)NH\(_2\)) and a carboxylic acid (RCOOH) on chemical degradation but only formula VI is in accord with all known reactions of mustard oil glucosides.
Raney nickel hydrogenation of sinigrin, tetramethylammonium glucosinalbate and the corresponding acetates is similar to well-known reactions of Raney nickel and requires no special comment.\textsuperscript{26,27} The production of a primary amine (RCH\textsubscript{2}NH\textsubscript{2}) demonstrates that the same carbon chain is present in the mustard oil glucoside. Consequently, mustard oils must be formed by rearrangement. The course of hydrogenolysis may be represented as follows:

\[
\begin{align*}
\text{(Ac)HO} & \quad \text{(Ac)HO} \\
\text{H} & \quad \text{H}
\end{align*}
\]

\[
\begin{align*}
\text{CH\textsubscript{2}OH} & \quad \text{C=N} \quad \text{OSO\textsubscript{3}} \\
\text{(Ac)HO} & \quad \text{RCH=NO\textsubscript{SO\textsubscript{3}}} \\
\text{RCH\textsubscript{2}NH\textsubscript{2}} & \quad \text{HSO\textsubscript{4}} \\
\end{align*}
\]

Mustard oil formation with concomitant liberation of glucose and sulfate by enzymatic hydrolysis is analogous to the Lossen rearrangement.\textsuperscript{33,34,35} The Lossen rearrangement, unlike the related Schmidt and Curtius reactions, generally proceeds in aqueous solution under mild conditions. An example of the Lossen rearrangement is the decomposition of benzoyl benzohydroxamate (X = X' = H). Renfrow

\[
\begin{align*}
\text{X-C\textsubscript{6}H\textsubscript{4}} & \quad \text{O} \quad \text{C-N-O-C-C\textsubscript{6}H\textsubscript{4}X'} \quad \text{O} \quad \text{H\textsubscript{2}O, 30°} \\
\text{0.1N NH\textsubscript{4}OH} & \quad \text{X-C\textsubscript{6}H\textsubscript{4}N=C=O} \\
& \quad \text{X-C\textsubscript{6}H\textsubscript{4}NH\textsubscript{2}NH\textsubscript{2}} \\
& \quad \text{X'\textsubscript{C\textsubscript{6}H\textsubscript{4}CO\textsuperscript{-}}}
\end{align*}
\]
and Hauser\textsuperscript{34,35} studied the effect of various substituents, \( X \) and \( X' \), on the rate of Losser rearrangement of this system. The study of a series of compounds varying \( X' \) only (\( X = H \)) and the application of the Hammett equation to the results gave a reaction constant, \( \rho' \), of 0.865.\textsuperscript{36} Similar experiments varying \( X \) (\( X' = H \)) yielded a \( \rho \) of -2.597. Thus electron-attracting substituents \( X' \) and electron-releasing substituents \( X \) increase the rate of this reaction. The magnitudes of the \( \rho \) and \( \rho' \)-values are especially significant. In order to explain the large value of \( \rho \) one must assume that migration of \( X-C_6H_4^- \) assists the loss of group \( C_6H_4O \cdot X' \). Thus, the Losser rearrangement of substituted benzoyl benzohydroxamic acids is a concerted process.

Failure of mustard oil glucoside acetates to be cleaved by myrosin\textsuperscript{30} suggests that the enzyme coordinates with the glucosyl portion of the molecule. Myrosin may then cause rupture of the glucosyl-sulfur bond, and the resulting fragment rearranges spontaneously to produce mustard oil.

Schneider's thicurethane glucosides and \( S-(\beta-d-l-(\text{thiogluco-pyranosyl})-phenylacetothiohydroximic acid are not attacked by myrosin, presumably because cleavage of the sulfur-glucosyl bond is too difficult. In view of the concerted nature of the Losser and related rearrangements,\textsuperscript{37,38} it appears likely that enzymatic cleavage of the glucosyl-sulfur bond in mustard oil glucosides is facilitated by simultaneous rearrangement and loss of sulfate. This concept requires that the sulfate and migrating groups (\( R \)) have the anti configuration, which may be represented as shown by
(1) in Table III. According to this interpretation, the glucoside-enzyme complex would be decomposed by water to yield initially $\beta$-D-glucose. That is, the reaction is one of double displacement: the enzyme displaces sulfur with inversion and then water enters in place of the departed sulfur and displaces the enzyme. $^{39}$ Mechanism (2) in Table III outlines this process. A previous experiment to determine the rotation of the initially liberated glucose failed because of the optical activity of the myrosin solution. $^{19}$

It should be noted, in the comparison of mustard oil glucosides and their N-hydroxy analogs, that the inductive effect of the sulfate group also will facilitate cleavage of the glucose-sulfur bond. Assignment of configuration on the basis of these considerations is not rigorous.

Mustard oil glucosides react with silver nitrate in aqueous solution to give insoluble silver salts with the liberation of $\alpha$-glucose. This is believed to be an electrophilic displacement on sulfur by silver ion with simultaneous nucleophilic attack of water on the glucosyl residue. This process, represented by (1), Table IV, occurs with inversion and is facilitated by the ability of the ether oxygen to stabilize the incipient carbonium ion. The decomposition of the silver derivatives of mustard oil glucosides to mustard oils appears closely analogous to mustard oil formation by enzymatic hydrolysis and is represented by (2), Table IV. Both processes appear to involve rearrangement of the same intermediate.

The alternate course of nitrile formation occurs when the intermediate (VIII) adds a proton. This protonated species IX
Table (III)

Enzymatic Hydrolysis of Mustard Oil Glucosides

1.
\[(\text{C}_6\text{H}_4\text{H}_2\text{H}_2\text{O}_5)_{2n} \rightarrow \text{H}_2\text{O} \]

2.
\[(\text{C}_6\text{H}_4\text{H}_2\text{H}_2\text{O}_5)_{2n} \rightarrow \text{Enzyme + } \text{C}_6\text{H}_12\text{O}_6 + \text{H}^+ \]

\[\text{Enzyme} + \text{C}_6\text{H}_12\text{O}_6 + \text{H}_2\text{O} \]

\[\text{Enzyme} + \text{C}_6\text{H}_12\text{O}_6 + \text{H}^+ \]

\[\text{Enzyme} + \text{C}_6\text{H}_12\text{O}_6 + \text{H}_2\text{O} \]

(Enzyme - Glucose Complex)

\[\beta\text{-d-Glucose} \]
corresponds to a free thiohydroxamic acid, and the unprotonated intermediate (VIII) is analogous to a thiohydroxamate salt. Thiohydroxamic acids have been observed to decompose readily into nitriles (see Section III). The decomposition of mustard oil glucoside silver salts to form nitriles is represented by (3), Table IV. The formation of nitrile and free sulfur, which has been detected in the enzymatic hydrolysis of sinigrin, may occur in a similar fashion by the action of the liberated acid on the enzyme-glucoside complex.

Phenylacetothiohydroxamic acid and its sodium salt (described in Section III) exhibit analogous reactions to silver derivatives of mustard oil glucosides. The free acid decomposes easily to benzylicyanide, and its sodium salt slowly undergoes rearrangement to produce initially benzyl isothiocyanate. In the case of phenylacetothiohydroxamic acid, the reactions of nitrile and isothiocyanate formation are characteristic of the free acid and salt respectively. It appears that the reactions of silver derivatives of mustard oil glucosides, which are capable of geometrical isomerism, to produce nitrile or mustard oil are characteristic of the charge or protonation of the particular intermediate involved, and may not necessarily be correlated with the configurations of the silver salt. Because the sulfate group is more electron-withdrawing than the hydroxyl group, silver derivatives of mustard oil glucosides are expected to decompose by either route at a much faster rate than unsulfonated thiohydroxamic acids. The existence of discrete intermediates in the decompositions of silver derivatives of mustard oil glucosides by either route may be questioned and, if such do exist, they would have a short life.
If one assumes the sulfate group to be anti to the migrating group and that nitrile formation occurs by cis elimination, both reactions may occur by a concerted mechanism.

The migrating group in the Lossen rearrangement retains its configuration. Formation of (±) L-sec-butyl mustard oil by the enzymatic hydrolysis of glucocochlearin is in agreement with this observation. Correlation of the configuration of the asymmetric carbon in (±) L-sec-butyl mustard oil and the corresponding carbon in natural isoleucine is of interest. From their correlation with (±)-2-methylbutyric acid, the two centers appear to have the same configuration, an observation which supports a biogenetic relationship between isoleucine and glucocochlearin.

Acid hydrolysis of sinigrin and sinalbin to hydroxylamine, hydrogen sulfide and a carboxylic acid (R-COOH) is analogous to the acid hydrolysis of hydroxamic acids and oxime-O-sulfonic acids. Free oxime-O-sulfonic acids were thermally unstable, easily undergoing a Beckmann rearrangement, but oxime-O-sulfonates were stable, crystalline solids. Acid hydrolysis of the oxime sulfonates afforded the oximes.

Hydrolysis of mustard oil glucosides to form a nitrile, which predominates in water or weak acid, undoubtedly competes with hydroxylamine formation in strong acid, accounting for the less than quantitative yields. The different reaction courses in weak or strong acid may be explained by the following proposals, which are outlined in Table V.
Table (V)

Acid Hydrolysis of Mustard Oil Glucosides

1. $\left(C_{6}H_{11}O_{5}\right)_{-S_{R}}C=NSO_{3}^{-}$

   $\overset{\text{A}}{\rightleftharpoons} \left(\left(C_{6}H_{11}O_{5}\right)_{-S_{R}}C=N\right)_{+}SO_{3}^{-}$

   $\overset{\text{H}_{2}O}{\rightarrow} RCOOH + H_{2}NOH + \text{Other Products}$

2. $\left(C_{6}H_{11}O_{5}\right)_{-S_{R}}C=NSO_{3}^{-}$

   $\overset{\text{B}}{\rightleftharpoons} \left(\left(C_{6}H_{11}O_{5}\right)_{-S_{R}}C=NH\right)_{-}SO_{3}^{-}$

   $\overset{\text{C}}{\downarrow}$

$\text{RCN} + S + \text{Other Products}$
Mustard oil glucosides (A) undergo rapid reversible acid-catalyzed isomerization to an isomer (B). The isomer (B) may decompose to nitrile by a rate-determining unimolecular process or be converted again to the conjugate acid (C). The rate of nitrile formation \( r_1 \) would be dependent on the concentration of species (B) and may be represented as:

\[
r_1 = k_1 \left[ B \right] = k_1 \left[ A \right]
\]

The rate of hydroxylamine formation \( r_2 \) is believed to be dependent upon the concentration of conjugate acid (C) and may be represented as follows:

\[
r_2 = k_2 \left[ C \right] \left[ H_2O \right] = k_2 \left[ A \right] \left[ H^+ \right] \left[ H_2O \right]
\]

Thus the ratio of hydroxylamine formation to nitrile formation \( r \) is dependent on acid concentration in the following way.

\[
r = \frac{k_2 \left[ A \right] \left[ H^+ \right] \left[ H_2O \right]}{k_1 \left[ A \right]} = k \left[ H^+ \right]
\]

Isomer (B) would be expected to decompose into nitrile more easily than (A) as trans-elimination can now occur.

Will and Korner\(^{10}\) found that sinigrin reacted with a small amount of barium hydroxide to form some mustard oil. The action of base on various \( \mathcal{S} \)-phenylglucosides and \( \mathcal{S} \)-phenylglucothiosides has been studied and found to proceed as follows to furnish 1,6-anhydroglucopyranose (levoglucosan)\(^{46,47}\).
The formation of mustard oil by the action of base on sinigrin may be readily explained by the same mechanism, which resembles the enzymatic cleavage.

Sinigrin has also been shown to hydrolyze in aqueous sodium hydroxide to produce thioglucose in small yield. To what extent these reactions compete is not known.

Sinigrin when treated briefly with anhydrous potassium methoxide solution yielded thioglucose and another product merosinigrin, formed from sinigrin by the loss of potassium bisulfate. Merosinigrin formed a triacetate, was stable in dilute acid and base and apparently
differed in the glucosyl residue from sinigrin as was indicated by the optical rotation, \( \alpha = \pm 149^\circ \). Schneider and Wrede proposed that merosinigrin was formed by the cyclization of the 2-hydroxyl group of the glucosyl residue to nitrogen with the loss of potassium bisulfate. Merosinigrin according to the revised formula for sinigrin may be:

\[
\text{Sinigrin and sinalbin have been shown to be disubstituted thiohydroximic acids. The general formula VI can be extended to all other mustard oil glucosides. The long known reactions of mustard oil glucosides to produce nitriles (RCN), mustard oils (RMCS), and carboxylic acids (RCOOH) as well as the new reactions of amine and hydroxylamine formation are in full agreement with this structure.}
\]
Experimental

Isolation of Sinigrin: One hundred grams of ground seed (in this case, brown mustard flour) was added to a boiling solution of 250 ml. of water and 750 ml. of acetone and the mixture boiled thirty to forty-five minutes. The suspension was filtered and the extract concentrated in vacuum to a volume of about 50 ml. The liquid was chilled, decanted from resin and clarified by extraction with ether (ca. three 20-ml. portions). The clear aqueous solution was now ready for ion exchange.

Amberlite IR-4B resin in a column 3-4 cm. in diameter was prepared for use by successive washes with 0.2 N potassium hydroxide, water and 0.5 N hydrochloric acid. The sequence of washes was performed thrice and after the final wash with acid the column (in chloride form) was washed well with water. A column containing 45 g. of "dry" resin, about 18 cm. high, will safely remove 10-15 g. of sinigrin, corresponding to 600-800 g. of flour; the capacity when saturated was about 21 g. of sinigrin. Sinigrin was eluted with 0.2 N potassium hydroxide (1.4-1.5 l. for 45 g. of resin) until the effluent became basic. The progress of the base front could be followed visually by the darkening* of the resin, and the appearance of base in the effluent was accompanied by a marked yellow coloration. The eluate was carefully neutralized with potassium hydroxide and evaporated in vacuum at 50° to dryness.

* Darkening was most apparent in re-used resin.
The residue was extracted with three or four portions of hot (ca. 100°) pyridine (total 50-100 ml. per 100 g. of flour) until the residue was colorless. The pyridine extract was evaporated in vacuum to leave essentially pure sinigrin as a colored, gummy residue. The sinigrin was dissolved in methanol (ca. 15 ml. per gram) and passed through a column of norite A (0.3-0.8 g. per g. of sinigrin) mixed with an equal volume of Celite. The column was washed with a little methanol and the colorless total effluent evaporated. The residue crystallized readily from 90% ethanol to yield pure sinigrin in yield of about 1.8% by weight of flour; m.p. 124-126°. A sample was recrystallized from 90% ethanol; m.p. 128-129°; [α]D 31.2° -17.27° (c = 9.8, water); λ max. 227 mμ (ε = 7,350, water).

Anal. Calcd. for C₁₀H₁₆₀₇S₂N₂K·H₂O: C, 28.88; H, 4.37; N, 3.37; S, 15.44; H₂O, 4.34.

Found: C, 29.03; H, 4.41; N, 3.48; S, 15.86; H₂O, 4.44.

Sinigrin lost one molecule of water when dried under 1 mm. pressure at ca. 77° for 24 hours. (Lit. 13, 23, 24.)

Rubidium Myronate: Sinigrin (160 mg.) in 10 ml. of water was passed slowly through a column of Amberlite IR-4B (1.5 g., 1 cm. diameter). After washing well with water, elution was accomplished with a solution of rubidium chloride (2 g. in 50 ml. of water). Evaporation of the effluent under reduced pressure, extraction of the residue with hot pyridine (thrice, 20-ml. portions) and evaporation of the pyridine in vacuum gave a white residue which was crystallized from ethanol-water to yield 217 mg. of rubidium myronate, m.p. 184-186°d (bath preheated to 180°); λ max. 227 mμ (ε = 7,330, water).
Ammonium, Cesium, Tetramethylammonium and Thallous Myronates: These salts were prepared by the use of Amberlite IR-120 cation exchange resin. One and seven tenths to three grams of resin (moisture content ca. 40%) in a 1-cm. diameter column was used for each preparation.

1. Ammonium Myronate: Three grams of resin was converted to the ammonium form by passing a solution of ammonium chloride (10 g. in 50 ml. of water) slowly through the column. After washing with water until the effluent was chloride-free, .5526 g. of sinigrin in 10 ml. of water was passed through the column followed by 4 ml. of water. The effluent was taken to dryness in vacuum and the residue crystallized from wet methanol-chloroform; yield .4413 g., m.p. 165-166° (bath preheated to 160°); λ max. 227 mμ (E = 7,330; water).

2. Tetramethylammonium Myronate: This material was prepared from .4605 g. of sinigrin and 3 g. of resin by the method outlined above. The resin was converted to tetramethylammonium form with tetramethylammonium chloride solution (10 g. in 50 ml. of water). Crystallization from wet methanol-chloroform yielded .2381 g. of tetramethylammonium myronate; m.p. 107-108°; λ max. 227 mμ (E = 7,460; water).
3. Cesium Myronate: Preparation was by the same general method from 1.621 g. of sinigrin and 1.7 g. of resin. The resin was prepared with a solution of cesium chloride (3.2 g. in 10 ml. of water). Cesium myronate crystallized from ethanol-water; yield 1.250 g.; m.p. 174-175° d (bath preheated to 168°); λmax. 227 mμ (ε = 7,290; water).

**Anal.** Calcd. for C10H16O9S2NCS: C, 24.45; H, 3.28;

**Found:**
C, 24.41; H, 3.37.

4. Thallous Myronate: Preparation was by the same method and scale as the cesium salt. The resin was prepared with a solution of thallous acetate (5 g. in 10 ml. of water). Thallous myronate crystallized from wet methanol-chloroform; yield 0.920 g.; m.p. 142-143° d (bath preheated to 135°); max. 222 mμ (ε = 9,620; water), displaced by absorption of the thallous ion. (Lit. 49)

**Anal.** Calcd. for C10H16O9S2NTl + H2O: C, 20.68; H, 3.12;

**Found:**

**Isolation of Sinalbin:** One hundred grams of yellow mustard flour was defatted with petroleum ether by four extractions, each with 200 ml. of stirred solvent during 10 minutes, and dried in air. The defatted flour (ca. 65 g.) was extracted twice by boiling 30 minutes in 250 ml. of 95% ethanol and filtered while hot. Sinalbin crystallized from the combined extracts on standing at room temperature overnight. Sinalbin, crystallized from 90% aqueous alcohol and dried over silica gel, melted at 102-103°, with δC2H5·10,9° (c=4.8, water), and had the composition of a solvate containing one molecule of ethanol and two molecules of water.

---

* Sinalbin did not crystallize in this laboratory until a seed was obtained, through the courtesy of Mr. R. W. King, R. T. French Company. Subsequent preparations have crystallized without seeding.
Anal. Calcd. for C_{30}H_{42}O_{15}N_{2}S_{2}+2H_{2}O+CH_{3}CH_{2}OH:  
C, 47.05; H, 6.42;  
N, 3.45; S, 7.52;  

Found:  
C, 47.01; H, 6.66;  
N, 3.43; S, 7.05.  

Sinalbin preparations equilibrated in air melted over a range of 
several degrees between 60 and 85°. An uptake in weight of .95 to 
2.78% accompanied the lowering of the melting point and was apparently 
dependent on the humidity. Conversion to the pentahydrate^{13} corresponds 
to a 1.10% weight increase.

The addition of .2668 grams of sinapine thiocyanate dissolved 
in 20 ml. of warm ethanol to 100 ml. of the filtrate from the 
sinalbin preparation yielded an additional .5435 g. of sinalbin, 
m.p. 60-65° (air dried), or 93% of theoretical corresponding to the 
sinapine thiocyanate added. (Lit. 13, 50, 51.)

Tetramethylammonium Glucosinalbate: One gram of sinalbin in 9.5 
ml. of water was treated with .4 g. of potassium thiocyanate in 
1 ml. of water and the resulting precipitate of sinapine thiocyanate 
was removed by centrifugation. After washing the precipitate with 
15 ml. of water, the combined aqueous solution was passed through a 
cation exchange column.

A column of Amberlite IR-120 was prepared from 15 g. of resin 
by washing slowly with 10% aqueous tetramethylammonium hydroxide 
solution until the effluent was basic. The column was washed with 
water until the effluent was neutral and the sinalbin solution was 
slowly passed through, followed by a wash with a little water. The 
eluate was evaporated to dryness in vacuum. The residue, in 15 ml.
of methanol, was decolorized with a little Norite and concentrated to ca. 5 ml. After the addition of 10 ml. of ethanol, tetramethylammonium glucosinalbulate crystallized; yield 4777 g. (78%); m.p. 187-188° d; (bath preheated to 180°). Recrystallization from methanol-ethanol yielded material of m.p. 191-192° d; [α]_D^{32} -18.86° (c = 3.3, water); max. 228 mμ (ε = 14,650) and 278 mμ (ε = 1600) in methanol.

**Anal.** Calcd. for C_{18}H_{30}O_{10}S_{2}N_{2}: C, 43.36; H, 6.07;

Found: C, 43.61; H, 6.20.

**Tetraacetyljosinigrin:** Sinigrin (1.0067 g.) was suspended in 4 ml. of dry pyridine and 3.8 ml. of acetic anhydride was added. After ca. 15 minutes, the mixture became semi-solid and was allowed to stand 3.5 hours at room temperature. Addition of 10 ml. of ether precipitated crude sinigrin tetraacetate which was washed with an additional 10 ml. of ether. Crystallization from ca. 80% aqueous ethanol yielded 1.1071 grams (81%) of sinigrin tetraacetate, m.p. 190-196° d, dependent on the rate of heating; [α]_D^{24} -17.25° (c=3.9, water); λ max. 221; mμ (ε = 7,120; methanol). (Lit. 30, 52)

**Preparation of Tetramethylammonium Pentaacetylglucosinalbulate:**

Tetramethylammonium glucosinalbulate (4777 g.) was mixed with 4 ml. of pyridine and 3.8 ml. of acetic anhydride and stirred 4 hours. The mixture became homogenous after about 20 minutes and was allowed to stand at room temperature 4 hours. Ether (40 ml.) was added, the mixture chilled 1 hour, and the solid removed by centrifugation. After crystallization from methanol-ethanol, tetramethylammonium pentaacetylglucosinalbulate melted at 178.5-179.8°; yield 5609 g.
(82%). Recrystallization from ethanol and drying under ca. 30 mm. for 12 hours yielded material melting at 161-162°; λ max. 221 mμ (ε = 11,900; methanol); [α]D 20-8° (C = 1.4, water). After drying 24 hours at 2mm., the material melted at 165-169°, and had the following composition.

**Anal.** Calcd. for C28H40O15N2S2: C, 47.45; H, 5.69;
Found: C, 47.05, 47.19;
H, 5.71, 5.74.

Material melting at 178° could not be obtained on recrystallization from ethanol.

**Preparation of Raney Nickel:** Fifty grams of Raney nickel alloy was dissolved in a solution of 90 g. of potassium hydroxide in 500 ml. of water at such a rate that the temperature remained about 50°. After the addition was complete, the mixture was stirred at this temperature for an additional 50 minutes. The nickel powder was washed with water by decantation until it was free of base and then stored under water in the cold (ca. 5°). (Lit. 28.)

**Hydrogenolysis of Sinigrin:** Hydrogenolysis experiments were all carried out in aqueous solution at room temperature.

1. An estimation of the rate of hydrogenolysis was made by treating a solution of 0.1 g. of sinigrin in 10 ml. of water with 0.3 g. of Raney nickel. After 15 minutes, a sample was diluted 1:500 with water and the optical density at 228 mμ was found to be .006, corresponding to less than .002 g. of sinigrin.

2. Sinigrin (.5310 g.) and 5 ml. of Raney nickel in water (total volume 25 ml.) was stirred at room temperature in a stoppered
flask for 40 minutes. Three grams of potassium hydroxide and 3 g. of Versene (ethylenediaminetetraacetic acid, used as the disodium salt) were added and the mixture was steam distilled into 1 ml. of concentrated hydrochloric acid, collecting ca. 175 ml. of distillate. This was taken to dryness in vacuum leaving a white residue. To this residue dissolved in 3 ml. of water was added 2 ml. of 10% aqueous sodium acetate and 1.2 ml. of p-nitrobenzoyl chloride solution (10% in benzene), and the mixture was stirred 4 hours. The mixture was extracted with 20 ml. of ether and the ether washed with small amounts of 5% sodium hydroxide, 3 N hydrochloric acid and water. The ether was taken to dryness and the residue crystallized from ethanol-water; yield 1350 g. (47%); m.p. 102-103°. A sample of the N-n-butyl-p-nitrobenzamide recrystallized for analysis from ethanol-water melted at 104-105°.

Anal. Calcd. for C_{11}H_{14}O_{3}N:  C, 59.44; H, 6.35;
          Found:  C, 59.29; H, 6.50.

The product from sinigrin did not depress the melting point of synthetic N-n-butyl-p-nitrobenzamide (m.p. 104.5-105.5°) and was identical by infrared spectrum to the synthetic material. N-n-propyl-p-nitrobenzamide (m.p. 101-102°) depressed the melting point of the n-butyl derivative (m.p. of mixture was 70-84°) and had a different infrared spectrum. (Lit. 53, 54)

3. Sinigrin (1.802 g.) and 6 ml. of Raney nickel in water (total volume 25 ml.) were stirred for one hour. Potassium hydroxide (3 g.) and 3 g. of Versene were added and the mixture was steam distilled into an excess of hydrochloric acid. The distillate was taken
to dryness and the residue was crystallized from acetone-ether; yield 0.0220 g. (17%); m.p. 208-208.5°, undepressed in mixture with synthetic \( \text{m} \)-butylamine hydrochloride. (Lit. 55)

To the acetone-ether mother liquor from the above experiment were added 5 ml. of 5% sodium acetate and 0.6 g. of \( \text{m} \)-nitrobenzenesulfonyl chloride. This mixture was stirred 16 hours, acidified, and the resulting precipitate crystallized from ethanol-water; m.p. 64-65°, identical by melting point of a mixture and infrared spectrum to synthetic \( \text{N} \text{-m} \text{-butyl-m-nitrobenzenesulfonamide} \). (Lit. 56)

**Hydrogenolysis of Tetramethylammonium Glucosinalbate:** The glucoside (0.4014 g.), 13 ml. of Raney nickel and water (total volume 30 ml.) were stirred 30 minutes at room temperature. The water was filtered from the catalyst and the catalyst washed with 10 ml. of water. This solution contained very little tyramine as evidenced by a very weak color with diazotized sulfanilic acid. Tyramine was displaced from the catalyst by stirring a few minutes in a Versene solution (50 ml. of water, 10 g. of Versene and sodium bicarbonate to neutralize). After filtering, this process was repeated once and then a second time using 20 g. of Versene. The Versene extracts were combined, the pH was adjusted to 9.85 (Beckman pH meter) by addition of sodium hydroxide, and the solution was extracted 24 hours with ether. Concentrated hydrochloric acid (1 ml.) was added to the ether and the mixture was taken to dryness. The residue, essentially pure tyramine hydrochloride, crystallized from ether-alcohol, melted at 263-265°; yield 0.0520 g. (37%). The melting point was undepressed by authentic tyramine hydrochloride and the sample gave an infrared spectrum identical to that of tyramine hydrochloride.
Tyramine hydrochloride (.0154 g.) from the hydrogenolysis of
tetramethylammonium glucosinalbate was dissolved in \( \frac{3}{2} \) ml. of 10% sodium hydroxide and 2 drops of benzoyl chloride were added. This solution was diluted with 3 ml. of water and, after standing 1 hour, the precipitated tyramine dibenzoate was removed by filtration. Crystallization from ethanol yielded .0160 g. (52%) of tyramine 0,N-dibenzoate, m.p. 170-171\(^\circ\), undepressed when mixed with an authentic sample. Synthetic tyramine dibenzoate and the product derived from hydrogenolysis had identical infrared spectra. (Lit. 55,57,58)

**Hydrogenolysis of Tetraacetylsinigrin:** Tetraacetylsinigrin (.4155 g.), 12 ml. of Raney nickel and water (total volume 35 ml.) were stirred 50 minutes at room temperature. The mixture was extracted with ether (4 times, 30-ml. portions), and the ether extracts were combined, dried with anhydrous magnesium sulfate and evaporated to dryness.
The resulting colorless gum was crystallized from ether-hexane with the help of a seed,* to give .0813 g. (34%) of polygalitol tetraacetate, m.p. 58-61\(^\circ\). After an additional crystallization, the product melted at 65.5-67.5\(^\circ\) with \([\alpha]^D_39\) 39.3\(^\circ\) (CHCl\(_3\), C=1.0). The material derived from sinigrin did not depress the melting point of an authentic sample* of polygalitol tetraacetate and had an infrared spectrum identical with that of polygalitol tetraacetate.

Tetraacetylsinigrin (.4934 g.) was treated as above. The polygalitol tetraacetate, obtained as a gum, was dissolved in 2 ml. of methanol and 5 ml. of dry methanol saturated with gaseous ammonia at

* Obtained through the courtesy of Dr. N.K. Richtmyer.
0° was added. This solution was allowed to stand at room temperature 4 hours, evaporated to dryness, dissolved in water and the water extracted with 5 ml. of ether. The water solution was taken to dryness and the polygalitol was crystallized from methanol, m.p. 134-136° with [α]$_D^{32}$ 45.3° (water, c = .83). The polygalitol, recrystallized from methanol, yielded .0345 g. (25%); m.p. 141.8-143.0°, undepressed in mixture with an authentic sample of polygalitol, and having an identical infrared spectrum to that of polygalitol. (Lit. 31, cf. 59)

**Hydrogenolysis of Tetramethylammonium Pentaacetylglucosinalbate:** The glucoside acetate (.2341 g.) was mixed with 5 ml. of Raney nickel and water (total volume 20 ml.) and stirred at room temperature 45 minutes. The mixture was extracted with ether (thrice, 20-ml. portions) and the combined ether extracts were dried first with saturated sodium chloride solution and then with magnesium sulfate. Removal of the ether yielded .0613 g. of clear gum which, crystallized from ether-petroleum ether, furnished .0367 g. (33%) of polygalitol tetraacetate; m.p. 67-68°, undepressed in mixture with an authentic sample. The infrared spectrum of the product from pentaacetylglucosinalbate was identical to that of polygalitol tetraacetate. (Lit. 31, 59)

**Detection of Hydroxylamine in the Acid Hydrolysates of Sinigrin and Tetramethylammonium Glucosinalbates:** Sinigrin (.0439 g.) in 1 ml. of concentrated hydrochloric acid was allowed to stand six hours at room temperature. A drop of this mixture on filter paper gave a positive test for hydroxylamine with picryl chloride and ammonia vapor, ammoniacal diacetyl monoxime-nickel salt reagent or with modified Csaky reagents, as described by Bremner. This hydrolysis mixture
and a solution of hydroxylamine hydrochloride as a control were chromatographed as an ascending chromatogram on Whatman No. 1 filter paper in a solvent mixture of 70 parts of methanol and 30 parts of 6 N aqueous hydrochloric acid until the solvent front had risen ca. 20 cm. The chromatograms were dried briefly at room temperature and treated with picryl chloride-ammonia or ammoniacal diacetyl monoxime-nickel salt exactly as described by Bremner to detect hydroxylamine. The control and sinigrin hydrolysate each gave spots of identical \( R_f \) (0.5 ± 0.05). Development by modified Csaky reagents was successful only if the chromatogram was previously sprayed with sodium acetate solution (35%). The chromatograms were prepared in a closed container without previous equilibration.

The acid hydrolysate of tetramethylammonium glucosinalbate, chromatographed in the same way and developed with picryl chloride-ammonia, produced a spot of identical \( R_f \) as a control of hydroxylamine hydrochloride. (Lit. 60, 61)

**Quantitative Determination of Hydroxylamine Produced by Acid Hydrolysis of Sinigrin, Tetramethylammonium Glucosinalbate and Related Compounds:** Hydroxylamine was quantitatively determined by the method of Csaky as modified by Yamada, and also by the method of Pucher and Day. The Yamada method is described below.

1 a. Assay procedure: To a sample containing ca. 0.08 mg. of hydroxylamine in 5-15 ml. of water was added 1 ml. of sulfanilic acid solution (1% in 30% acetic acid) and \( \frac{1}{2} \) ml. of iodine solution (1.3% in glacial acetic acid). After exactly 3 minutes,
1 ml. of sodium arsenite solution (2% in water) and 1 ml. of 
c-1-naphthylamine solution (.3% in 30% acetic acid) were added. 
Water was added to bring the total volume to 25 or 50 ml. and 
the density of the resulting cherry-red solution was measured 
at 500 m\(\mu\) on a Beckman model DU spectrophotometer, using water 
as a blank. Neglect of a reagent blank caused no significant 
error. All reagents were measured from micro burettes. By 
assaying standard hydroxylamine solutions the following data, 
used to plot a calibration curve, was obtained. Baker's 
hydroxylamine hydrochloride, 99.9% pure, which had been dried 
1 hour at 110\(\degree\) was used. Beer's law was obeyed in the concen-
tration range studied.

<table>
<thead>
<tr>
<th>H(_2)NOH(\cdot)HCl (mg.)</th>
<th>D (500 m(\mu))</th>
<th>Total Volume (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>.0214</td>
<td>.392</td>
<td>25</td>
</tr>
<tr>
<td>.0214</td>
<td>.196</td>
<td>50</td>
</tr>
<tr>
<td>.0512</td>
<td>.830</td>
<td>25</td>
</tr>
<tr>
<td>.0512</td>
<td>.432</td>
<td>50</td>
</tr>
<tr>
<td>.0512</td>
<td>.208</td>
<td>100</td>
</tr>
<tr>
<td>.0512</td>
<td>.086</td>
<td>250</td>
</tr>
<tr>
<td>.0314</td>
<td>.579</td>
<td>25</td>
</tr>
</tbody>
</table>

b. Hydrolysis procedure: The glucoside (.0020 to .0050 g.), 
\(\frac{1}{2}\) to 2 ml. of 2\% 2,4 dinitrophenylhydrazine in 6 M sulfuric acid, 
and water to give the desired acid strength were heated on a steam 
bath, usually for two hours. This solution was diluted to 15 ml. 
with water and centrifuged to remove a red precipitate. A suitable 
portion of the solution (3 to 5 ml.) was removed and prepared for 
analysis by extracting thrice with 3 ml. of 10\% ethyl acetate in 
benzene, 3 times with 3 ml. of benzene, neutralizing to Congo Red 
with 35\% sodium acetate solution and extracting again thrice with
3 ml. of benzene. The resulting colorless solution was assayed as previously described and the density at 500 m measured against water. The quantity of hydroxylamine was determined by reference to the calibration curve, and the theoretical yield from the glucoside calculated on the basis of the following equation:

\[
\text{S-} \begin{array}{c} \text{C}_6\text{H}_{11}\text{O}_5 \\ \text{R-O=NO}_3 \end{array} \xrightarrow{\text{H}_2\text{O}} \text{H}_2\text{NOH} + \text{RCOOH} + \text{H}_2\text{SO}_4 + \text{other products} \\
\text{H}^+
\]

Neglect of blanks introduced no significant error. The results of this procedure using sinigrin, tetramethylammonium glucosinolate and various compounds described in Section III are given in Table VI. (Lit. 29a, 61)

2. Pucher-Day procedure: To the sample of hydroxylamine in a volumetric flask were added 2 drops of benzoyl chloride, 2 ml. of 2% aqueous sodium acetate solution and 4 ml. of ethanol. After three minutes 2 ml. of 0.5% aqueous ferric chloride solution was added and the mixture diluted to an appropriate volume with water. The density at 520 m\(\mu\) of the resulting violet solution was measured against a blank. Using standard hydroxylamine hydrochloride solution the following data were obtained.

<table>
<thead>
<tr>
<th>(\text{H}_2\text{NOH}\cdot\text{HCl} \text{ (mg.)} )</th>
<th>Volume</th>
<th>(D_{520} \text{ m} \mu )</th>
</tr>
</thead>
<tbody>
<tr>
<td>.506</td>
<td>10 ml.</td>
<td>.738</td>
</tr>
<tr>
<td>.190</td>
<td>10 ml.</td>
<td>.280</td>
</tr>
</tbody>
</table>
Table (VI)
Results of Hydroxylamine Assay

1. Yamada procedure

<table>
<thead>
<tr>
<th>Glucoside</th>
<th>Acidity ($\text{M}_2\text{SO}_4$)</th>
<th>Yield of $\text{H}_2\text{NOH}$(%)</th>
<th>Number of Determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinigrin</td>
<td>6 $\text{M}$</td>
<td>51</td>
<td>2</td>
</tr>
<tr>
<td>Sinigrin</td>
<td>5</td>
<td>51</td>
<td>1</td>
</tr>
<tr>
<td>Sinigrin</td>
<td>4</td>
<td>59</td>
<td>2</td>
</tr>
<tr>
<td>Sinigrin</td>
<td>3</td>
<td>56</td>
<td>4</td>
</tr>
<tr>
<td>Sinigrin</td>
<td>2</td>
<td>46</td>
<td>1</td>
</tr>
<tr>
<td>Sinigrin</td>
<td>1</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Sinigrin</td>
<td>4*</td>
<td>67</td>
<td>1</td>
</tr>
<tr>
<td>Tetramethylammonium glucosinabate</td>
<td>4</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>Sinalbin</td>
<td>4</td>
<td>51</td>
<td>1</td>
</tr>
<tr>
<td>Tetramethylammonium glucotropaeololate</td>
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<td>71</td>
<td>1</td>
</tr>
<tr>
<td>Potassium tetraacetylglucotropaeololate</td>
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<td>65</td>
<td>1</td>
</tr>
<tr>
<td>S-$\beta$-$d$-l-(Tetraacetylglucopyranosyl)-phenylacetothiohydroximic acid</td>
<td>4 $\text{M}$</td>
<td>49</td>
<td>1</td>
</tr>
<tr>
<td>S-$\beta$-$d$-l-Glucopyranosylphenylacetothiohydroximic acid</td>
<td>4 $\text{M}$</td>
<td>36</td>
<td>2</td>
</tr>
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</table>

*Allowed to stand 41 hours at room temperature.

2. Pucher-Day procedure

<table>
<thead>
<tr>
<th>Acidity ($\text{M}_2\text{HCl}$)</th>
<th>Hydrolysis Conditions</th>
<th>Yield of $\text{H}_2\text{NOH}$(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12 hours at room temperature</td>
<td>31</td>
</tr>
<tr>
<td>12</td>
<td>30 hours at room temperature</td>
<td>46</td>
</tr>
<tr>
<td>1</td>
<td>3 hours on a steam bath</td>
<td>34</td>
</tr>
<tr>
<td>1</td>
<td>6 hours on a steam bath</td>
<td>34</td>
</tr>
</tbody>
</table>
Sinigrin was hydrolyzed with hydrochloric acid under various conditions of time, temperature and acidity. The samples were carefully neutralized to litmus with 0.2 N potassium hydroxide and assayed as described above. The yields of hydroxylamine were calculated by use of the data obtained with the standards and are recorded in Table VI. (Lit. 29b)

Isolation of Hydroxylamine from Acid Hydrolysis of Sinigrin and Tetramethylammonium Glucosinalbate as Fluorenone Oxime:

1. Sinigrin (.5450 g.) was hydrolyzed in a solution of 0.52 g. of 2,4 dinitrophenylhydrazine in 1.67 ml. of concentrated sulfuric acid and water to make 10 ml. by heating on a steam bath 2 hours. After cooling, the mixture was filtered from a red precipitate and extracted twice with 10% ethyl acetate (20-ml. portions) in benzene. Sodium acetate* (7.35 g.) was dissolved in the mixture causing an additional red precipitate. After removal of the solid by filtration, this solution was added to 0.4 g. of fluorenone in 10 ml. of methanol. Addition of 10 ml. of water produced a homogeneous solution when heated on a steam bath. Warming on a steam bath was continued 1.5 hours after which the mixture was refluxed 1.5 hours with an electric heating-mantle. The mixture was then chilled and the crude mixture of fluorenone and fluorenone oxime was removed by filtration. This solid, dried and crystallized from petroleum ether-benzene and then twice from

* The optimum pH for oxime formation is ca. 5.4 (Lit. 63) which requires more sodium acetate. Solution difficulties would be increased by more sodium acetate.
benzene, furnished .0331 g. (13%) of pure fluorenone oxime, m.p. 191-192°, unde pressed in mixture with an authentic sample. The product derived from sinigrin and synthetic fluorenone oxime gave identical infrared spectra.

2. Tetramethylammonium glucosinalbate (.2622 g.) was hydrolyzed under the same conditions used in the preceding experiment. After cooling, extracting and partially neutralizing with sodium acetate (7.35 g.) and filtering as in the preceding experiment, the solution was extracted twice with benzene (20-ml. portions). The aqueous solution was added to .8 g. of fluorenone in 10 ml. of methanol and, after the addition of 15 ml. of water, the mixture became nearly homogeneous at the reflux temperature. The solution was refluxed 3 hours, cooled, extracted twice with ether (50-ml. portions) and the combined ether solutions were evaporated to dryness. The residue was dissolved in benzene (50 ml.) and concentrated to ca. 5 ml. This solution was placed on a column of alumina (activated 1/2 hour at 360°; 3 g. slurried in benzene). Washing the alumina with 50 ml. of benzene removed all of the fluorenone. Washing with 20 ml. of ether and evaporation of the ether yielded .0480 g. (47%) of fluorenone oxime, m.p. 189-191°, unde pressed in mixture with a synthetic sample. Identical infrared spectra were given by synthetic fluorenone oxime and the product derived from tetramethylammonium glucosinalbate. (Lit. 62, 63)

**Synthetic p-Bromophenacyl Vinylacetate**: Vinylacetic acid (.0914 g.) was neutralized with 10% aqueous sodium hydroxide and then just acidified to litmus with dilute hydrochloric acid.
p-Bromophenacyl bromide (.2594 g.) in 3 ml. of ethanol was added, the mixture refluxed one hour, cooled and extracted with ether (50 ml.). The ether was evaporated to dryness and the residue was dissolved in .5 ml. of benzene and petroleum ether added to make 10 ml. This solution was chromatographed on alumina (3 g., Activity III, washed with ethyl acetate). p-Bromophenacyl bromide was washed from the column with petroleum ether and pure p-bromophenacyl vinylacetate was eluted with 20% benzene-petroleum ether; yield .1210 g. (40%); m.p. 58-58.5°. One crystallization from water-ethanol yielded material melting at 61-62°, unchanged by additional crystallizations.

Anal. Calcd. for C_{12}H_{11}O_3Br: C, 50.90; H, 3.92;

Found: C, 51.09; H, 4.15.

Subsequent preparations melted at about 50° on crystallizing from aqueous alcohol but after drying under oil pump vacuum melted at 61°. (Lit. 64, 65)

p-Bromophenacyl Vinylacetate from Sinigrin Hydrolysis: Sinigrin (.4296 g.) in 4 ml. of 3 M sulfuric acid was heated two hours on a steam bath. The mixture was extracted twice with ether (50-ml. portions). The ether was carefully neutralized with 10% aqueous sodium hydroxide and acidified to litmus with a drop of dilute hydrochloric acid. The ether was removed, 1 ml. of water and an ethanolic solution of p-bromophenacyl bromide (.2968 g. in 4 ml.) added and the mixture refluxed one hour. The cooled mixture was extracted with ether (50 ml.) and the ether evaporated to dryness. Chromatography of the material as previously described furnished
.0712 g. (24%) of \( p \)-bromophenacyl vinylacetate, m.p. 58.5°, which melted at 60-61° after recrystallizing from water-ethanol. This material was identical by melting point of mixture and infrared spectrum to the synthetic \( p \)-bromophenacyl vinylacetate.

Synthetic \( p \)-Phenylphenacyl Vinylacetate: One half gram of vinylacetic acid, neutralized with 10% sodium hydroxide and acidified with dilute hydrochloric acid, was refluxed with .5 g. of \( p \)-phenylphenacyl bromide in 10 ml. of ethanol for 1½ hours. On cooling crystals formed, m.p. 65-67°. Two recrystallizations from methanol-water gave material equal in purity to chromatographed material; yield .2260 g. (44% from bromide), m.p. 76-77°.

Anal. Calcd. for \( C_{18}H_{16}O_3 \): C, 77.12; H, 5.75;

Found: C, 76.85; H, 5.70.

\( p \)-Phenylphenacyl Vinylacetate from Sinigrin Hydrolysis: Sinigrin (.4569 g.) in 3 ml. of 3 N sulfuric acid was heated two hours on a steam bath, cooled, extracted with ether and neutralized in the usual way. The ether was removed, ca. 2 ml. of water and a solution of \( p \)-phenylphenacyl bromide (.2 g. in 5 ml. of ethanol) added and the mixture refluxed 1½ hours. The mixture was cooled and the resulting solid removed by filtration. Chromatography of this material on alumina (Activity III as previously described) furnished .0376 g. (18% from bromide) of \( p \)-phenylphenacyl vinylacetate, which, after crystallization from water-methanol, melted at 75-76°. A mixture with the synthetic compound melted at 76-76.5°, and the infrared spectra were identical.
p-Bromophenacyl and p-Phenylphenacyl Crotonates: These derivatives were prepared in the usual way from .5 g. of acid and .5 g. of bromide. The previously unknown p-phenylphenacyl ester crystallized from water-alcohol, yield .3688 g. (73% from bromide), m.p. 105-106°.

Anal. Calcd. for C₁₈H₁₆O₃: C, 77.12; H, 5.75;

Found: C, 77.12; H, 5.73.

The p-bromophenacyl ester, obtained in the same way, melted at 95.5-96.8°. These materials depressed the melting points of the corresponding vinylacetic esters and had different infrared spectra.

(Lit. 66)

p-Hydroxyphenylacetic Acid and Hydroxylamine from Acid Hydrolysis of Tetramethylammonium Glucosinolate: The glucoside (.3867 g.) was heated with 3 ml. of 3 N hydrochloric acid on a steam bath 1½ hours. The cooled hydrolysis mixture was extracted with ether three times (10-ml. portions) and the combined ether extracts were taken to dryness. (The water phase was saved for a following experiment.) The semi-crystalline yellow residue was dissolved in 4 ml. of ether and passed through a column of Norite A (1/2 g. Norite mixed with an equal amount of Celite in a 0.8 cm. diameter column). After washing the column with a small amount of ether, the colorless filtrate was evaporated to dryness. The residue, crystallized from benzene, furnished .0180 g. (41%) of p-hydroxyphenylacetic acid, m.p. 148-149°. After an additional crystallization from benzene, the material melted at 150.4-151.2°, identified by melting point of a mixture and infrared spectrum with synthetic p-hydroxyphenylacetic acid.
The water phase from the preceding experiment was neutralized to Congo Red with solid sodium acetate. This solution was added to a refluxing solution of fluorenone (0.7 g. in 10 ml. of methanol) and refluxed 4 hours. The cooled mixture was extracted with ether (twice, 30-ml. portions) and the ether evaporated to dryness. The residue was chromatographed as previously described. Fluorenone oxime, 0.0082 g. (5%), m.p. 186-189°, was isolated and identified by its infrared spectrum. (Lit. 65)
Section III
The Synthesis of Benzyl Mustard Oil Glucoside;

The Glucotropaeolate Ion

The reported preparation of benzothiohydroxamic acid and its conversion to a stable S-benzyl derivative suggested a synthetic route to the mustard oil glucosides.

Benzothiohydroxamic acid (I) was obtained as an unstable, impure oil by the action of hydroxylamine on dithiobenzoic acid. This material could be alkylated with benzyl chloride and potassium carbonate to furnish S-benzylbenzothiohydroximic acid (II), a stable, crystalline material which was obtained in pure condition. It is of interest to note that only one of the two theoretically possible isomers was obtained by Cambi. The alkylation reaction of benzothiohydroxamic acid resembles alkylation of thioamides to produce imino thioethers (III) rather than the monoalkylation of simple hydroxamic acids (which occurs on the oxygen attached to nitrogen), and presumably reflects the great nucleophilic character of divalent sulfur.

Glucotropaeolin (IV), a natural precursor of benzyl mustard oil which had recently been isolated as a crystalline tetraacetate, was selected for synthesis. The synthetic route, implicit in the work of Cambi, was to alkylate phenylacetothiohydroxamic acid with acetobromoglucose and sulfonate the resulting S-β-d-1-(tetra-acetylglucopyranosyl)-phenylacetothiohydroximic acid to furnish
the tetraacetylglucotropaeolate ion or its geometrical isomer. Acetobromoglucose has long been used to synthesize glucosides and thiogluco- 
sides\textsuperscript{71} by this type of reaction, known as the Königs- 
Knorr reaction.\textsuperscript{72} This reaction is a bimolecular displacement and 
yields the $\beta$-series from $\alpha$-acetobromoglucose by inversion.

The dithio acid required for this synthesis, dithiophenylacetic 
acid, was easily prepared by the addition of benzyl magnesium 
chloride to an excess of carbon disulfide.\textsuperscript{73} The ethereal dithio-
phenylacetate thus obtained reacted rapidly with aqueous hydroxyl-
amine hydrochloride in the cold to furnish crystalline phenylacetothio-
thiohydroxamic acid (V), the first simple thiohydroxamic acid to 
be obtained analytically pure.

\[
\begin{align*}
\text{III} & \quad \text{IV} \\
\text{V} & 
\end{align*}
\]

Benzothiohydroxamic acid was reported by Cambi to decompose 
easily into benzonitrile, sulfur and water. Similarly, phenylacetothio-
thiohydroxamic acid decomposed into benzyl cyanide, sulfur and water.
The material smelled faintly of benzyl cyanide even when freshly 
crystallized and decomposed completely after a few days at room 
temperature, but appeared completely stable below 0°. Heating in 
bulk to ca. 100° caused rapid, exothermic decomposition into the 
same products. Solutions of the thiophydroxamic acid in chloroform 
or ether were moderately stable, but a methanolic solution de-
posited sulfur quantitatively during 24 hours at room temperature.
In contrast to the free acid, sodium phenylacetothiohydroxamate was moderately stable at room temperature. However, during ten weeks at room temperature it produced N,N'-dibenzylthiourea by Lossen rearrangement. Benzyl isothiocyanate, the initial product, partially hydrolyzed to benzylamine which reacted with unchanged benzyl isothiocyanate to form the thiourea. As was noted in Section II, the decomposition of the thiohydroxamic acid to nitrile and the rearrangement of the thiohydroxamate to isothiocyanate closely resemble reactions of silver derivatives of mustard oil glucosides. The Lossen rearrangement of phenylacetothiohydroxamic acid establishes a point of similarity between thiohydroxamic acids and their oxygen analogs, but the decomposition of the thiohydroxamic acid to nitrile, sulfur and water appears to have no counterpart among hydroxamic acids or thioamides.

Phenylacetothiohydroxamic acid has a single ultraviolet maximum at 267 mµ (log ε ca. 3.9) in methanol. Thioacetamide has a similar maximum at 267 mµ (log ε 4.01) in ethanol.74 The hydroxyl group of the thiohydroxamic acid, which is not directly part of the chromophore, appears to have almost no effect on the ultraviolet absorption.

Wuyts and collaborators75 found that nitriles and also oximes as well as free sulfur were formed by the reaction of hydroxylamine with dithio acids in warm pyridine solution. Formation of oxime was appreciable only with aromatic dithio acids. For example, dithio-α-naphthoic acid reacted with hydroxylamine to yield 88%
of the oxime, whereas dithiophenylacetic acid furnished only 12% of the corresponding oxime. Thiohydroxamic acids were presumably intermediates and decomposed under the condition of the reaction to give the products indicated. Because of the meager data available concerning thiohydroxamic acids, the details of their decompositions are uncertain. The mechanisms in Table VII are proposed as reasonable explanations for these transformations.

The suggestion that thiohydroxamic acids decompose by the cyclic process 1-A explains in part the easy decomposition of phenylacetothiohydroxamic acid in the solid state. This mechanism is reminiscent of those proposed for the Chugaev and amine oxide eliminations. A process such as 1-B probably occurs in hydroxylic solvents. However, in polar aprotic solvents the molecule may be solvated so as to interrupt both processes.

Oxime formation (2) by the decomposition of a thiohydroxamic acid is presumably determined by the ability of the group (R) to stabilize an adjacent negative charge. Consequently, only aromatic dithio acids or other special types will react extensively in this way. The rearrangement of the thiohydroxamate (3) is analogous to the well-known Lossen rearrangement.

Phenylacetothiohydroxamic acid reacted with acetobromoglucose and potassium hydroxide to furnish S-(β-d-1 (tetraacetylglucopyranosyl)-phenylacetothiohydroxamic acid (VI). Only one isomer was obtained. This material, in contrast to phenylacetothiohydroxamic acid, was completely stable and had no accessible ultraviolet maximum. Hantzsch observed a similar change in the
Table (VII)

Decompositions of Thiohydroxamic Acids

1-A
\[
\begin{aligned}
S^+ & \quad R-C-NHOH & \quad \leftrightarrow & \quad SH & \quad R-C-NHOH & \quad \rightarrow & \quad [ & \quad \begin{array}{c}
\text{S-H} \\
\text{R-C-NHOH}
\end{array} & \quad \rightarrow & \quad RCN + S + H_2O
\end{aligned}
\]

1-B
\[
\begin{aligned}
S^+ & \quad R-C-NHOH & \quad \leftrightarrow & \quad SH & \quad R-C=NHOH & \quad \rightarrow & \quad [ & \quad \begin{array}{c}
\text{S-H} \\
\text{R-C=NHOH}
\end{array} & \quad \rightarrow & \quad RCN + S + H_2O
\end{aligned}
\]

2.
\[
\begin{aligned}
SH & \quad R-C=NHOH & \quad \rightarrow & \quad [ & \quad \begin{array}{c}
\text{S-H} \\
\text{R-C=NHOH}
\end{array} & \quad \rightarrow & \quad R-C=NHOH + S & \quad \rightarrow & \quad R-CH=NHOH
\end{aligned}
\]

3.
\[
\begin{aligned}
[ & \quad S^- \\
R-C=NHOH & \quad \leftrightarrow & \quad [ & \quad S^- \\
R-C-\bar{N}OH & \quad \rightarrow & \quad [ & \quad S^- \\
R-C-\bar{N}...OH & \quad \rightarrow & \quad RNCS + NaOH
\end{aligned}
\]
ultraviolet spectrum of thioacetamide when it was converted to its S-methyl ether. These observations imply that free thiohydroxamic acids, as well as free thioamides, exist mainly with a carbon-sulfur double bond. This conclusion is supported by the presence of a N-H and the absence of a S-H band in the infrared spectrum of phenylacetothiohydroxamic acid.

![Chemical Structure]

S-\(\text{d-1}\)-(Tetraacetylglucopyranosyl)-phenylacetothiohydroximic acid was sulfonated with sulfur trioxide-pyridine\(^6\) to furnish the tetraacetylglucotropaeolate ion, which was isolated as crystalline tetramethylammonium, sodium and potassium salts. The sodium and potassium salts were identical to the corresponding naturally derived materials. Ammonolysis of tetramethylammonium tetraacetylglucotropaeolate furnished crystalline tetramethylammonium glucotropaeolate, identical with naturally derived material in all respects. The synthetic glucoside was cleaved rapidly and quantitatively by myrosin solution to furnish benzyl isothiocyanate. By contrast, the unsulfonated analog of the glucotropaeolate ion, S-\(\text{d-1}\)-glucopyranosylphenylacetothiohydroximic acid, obtained by ammonolysis of VI, was not cleaved by myrosin at a detectable rate. No crystalline material could be obtained by ammonolysis of the sodium and potassium salts.

The glucotropaeolate ion has no accessible ultraviolet maximum, in
contrast to most mustard oil glucosides, which have a maximum at about 228 m\(\mu\) in water.\(^7\) The ultraviolet spectra of the glucotropaeolate ion and the tetraacetylglucotropaeolate ion are nearly identical. Each is a composite derived from the benzyl chromophore and the imino thio-ether chromophore, such that the imino thioether band is obscured. It is of interest to compare the ultraviolet spectrum of the tetraacetylglucotropaeolate ion with that of the glucosinalbate and the pentaacetylglucosinalbate ions. The conversion of the phenolic hydroxyl group of the glucosinalbate ion to an acetoxy group, because of the weaker conjugating power of the acetoxy group, produces a chromophore which more closely resembles the benzyl group. For example, the ultraviolet band of phenol at ca. 270 m\(\mu\) (\(\varepsilon = 2,000\)) appears at about the same position for phenylacetate but with an extinction of only ca. 250, a value close to that for the benzyl group.\(^7\) Hence, the ultraviolet spectrum of tetramethylammonium pentaacetylglucosinalbate, in contrast to the free glucoside, has no prominent absorption near 280 m\(\mu\). While the imino thioether band appears in the spectrum of tetramethylammonium pentaacetylglucosinalbate, the resemblance to the spectrum of potassium tetraacetylglucotropaeolate is apparent. These spectra are compared in Figure I.

The synthesis of the glucotropaeolate ion proceeded in ca. 8% overall yield from benzyl chloride. As no effort was made to achieve maximum yield, this can probably be improved. Only one of the two theoretically possible isomers was obtained from the reaction of phenylacetonothiohydroxamic acid with acetobromoglucose. Whether or not this corresponds in configuration to the mustard oil glucoside is not known at the present time.
Figure 1
Comparison of Ultraviolet Spectra

1. Potassium Tetraacetylglucotropaeolate
2. Tetramethylammonium Pentaacetylglucosinolate
3. Tetramethylammonium glucosinolate
This synthesis of the glucotropaclate inn is presumed to be a general one, capable of extension to other members of the series. It is limited by the accessibility of thiohydroxamic acids of general formula VII. At the present time it appears that only two of these

\[
S \quad R-C-NH\text{OH}
\]

VII

compounds, benzothiohydroxamic and phenylacetothiohydroxamic acids, have been prepared. The constitution of a substance reported to be isonicotinothiohydroxamic acid\textsuperscript{80} seems to the author to be uncertain. This material was prepared from potassium dithioisonicotinate and hydroxylamine in aqueous solution by refluxing the mixture six hours, conditions that would rapidly decompose phenylacetothiohydroxamic and benzothiohydroxamic acids. Preparation of thiohydroxamic acids by methods other than from dithio acids may be necessary, as only a few dithio acids have been prepared in satisfactory yield.\textsuperscript{81}
Experimental

Phenylacetothiohydroxamic Acid: Benzyl chloride (37.5 ml., .33 mole) in 100 ml. of dry ether was added to 8.25 g. of magnesium (.34 mole) in 100 ml. of dry ether under a nitrogen atmosphere at such a rate that the ether refluxed rapidly. The mixture was stirred vigorously during addition of benzyl chloride and the stirring continued ½ hour after addition was complete. This freshly prepared benzyl Grignard reagent was slowly added to a cold (0°) solution of 30.5 ml. of carbon disulfide (.506 mole) in 150 ml. of ether. This operation was performed under nitrogen and the mixture was rapidly stirred with a magnetic stirrer. Addition of the benzyl Grignard reagent to the carbon disulfide solution required about one hour. The ether solution of magnesium dithiophenylacetate was allowed to stand overnight at room temperature. (Lit. 73)

The ethereal dithiophenylacetate solution was cooled in ice and stirred rapidly while 15 g. of hydroxylamine hydrochloride in 150 ml. of ice water was added. Hydrogen sulfide was vigorously evolved and the rate of addition of hydroxylamine had to be controlled so that the reaction could be contained. The addition required 10 minutes and the two-phase mixture was stirred five minutes longer. The ether phase (dark red) was separated from the water phase (milky yellow, pH ca. 5) and the water was acidified to Congo Red and extracted with 100 ml. of ether. The combined ether extract was concentrated under water pump vacuum to remove excess hydrogen sulfide and extracted with a cold (0°)
solution of potassium carbonate (20 g. in 100 ml. of water). Acidification of the potassium carbonate extract to Congo Red with cold 10% sulfuric acid produced a large quantity of paste-like solid, which was removed by ether extraction (two 100-ml. portions). The potassium carbonate extraction sequence was repeated (4 g. in 50 ml. of water) and the ether solution obtained combined with the previous ether extract. The combined ether solution was dried over anhydrous magnesium sulfate, filtered and concentrated in vacuum to ca. 50 ml. Benzene (100 ml.) was added and the solution was again dried with magnesium sulfate. After filtering and washing the magnesium sulfate with benzene (100 ml.), the volume was reduced in vacuum to ca. 125 ml. (Concentrations were all at room temperature or below. Most of the ether must be removed or the product will not crystallize.) Hexane (200 ml.) was added and large, colorless crystals of phenylacetothiohydroxamic acid began forming. After 30 minutes 100 ml. of hexane was added and the mixture chilled. Phenylacetothiohydroxamic acid, obtained in 33% yield (18.2 g.) after filtering and drying 30 minutes under vacuum, melted at 73-75.5° without apparent decomposition. Phenylacetothiohydroxamic acid had a single ultraviolet maximum at 267 m\u00b5 in methanol (log\vspace{3pt} \text{E} \text{ ca.} 3.9) which, in .01 N methanolic sodium hydroxide (25 ml. of 0.1 N aqueous sodium hydroxide diluted to 250 ml. with methanol), was shifted to 247 m\u00b5 (log\vspace{3pt} \text{E} \text{ ca.} 3.8). The extinctions in methanol could not be measured accurately as the thiohydroxamic acid decomposed. Phenylacetothiohydroxamic acid could be stored below 0° but decomposed slowly at room temperature. The material produced an intense violet color with ferric chloride, similar to that produced by simple hydroxamic
acids. Portions of phenylacetothiohydroxamic acid, recrystallized for analysis, melted sharply although the temperature ranged from 72 to 75° (Lit. cf. 40)

**Anal.** Calcd. for C₆H₃ONS: C, 57.46; H, 5.42; N, 8.38; S, 19.17;

**Found:** C, 57.61, 57.70; H, 5.43, 5.51;

N, 8.25; S, 18.94.

**Decomposition of Phenylacetothiohydroxamic Acid:**

1. The acid (.1072 g.) was placed in a Craig recrystallizing tube and dissolved in ½ ml. of methanol. After standing 2½ hours at room temperature, yellow crystals had been deposited. The methanol was removed and the solid, after drying, weighed .0207 g. The solid was identified as sulfur by its X-ray powder diffraction pattern, melting point (111-114°) and mixed melting point with sulfur (116-117°). A sample, submitted for analysis, was found to be 98.48% sulfur, corresponding to 18.94% from phenylacetothio-

hydroxamic acid.

2. Phenylacetothiohydroxamic acid (.5 g.) was heated in a Woods metal bath. At bath temperature ca. 98°, a spontaneous, exothermic reaction occurred forming a solid and a liquid. Ethyl alcohol (5 ml.) was added to the mixture and the liquid removed from the solid by centrifuging. The liquid was fractionated under vacuum to yield benzyl cyanide, identified by infrared spectrum and refractive index (nD 30 1.5185).

The solid was dissolved in carbon disulfide and crystallized on evaporation to yield .2818 g. (18.8%) of yellow sulfur crystals.

3. Samples of phenylacetothiohydroxamic acid all smelled more or less of benzyl cyanide, and after about a day at room temperature,
had become partially liquid. Solutions of phenylacetothiohydroxamic acid in anhydrous ether or chloroform were stable for at least 24 hours but methanolic solutions rapidly decomposed as judged by periodic examination of the ultraviolet band. (cf. 40, 75)

**Sodium Phenylacetothiohydroxamate:** The thiohydroxamic acid (0.9946 g.) in ether was treated with an ethereal sodium methoxide solution (0.133 g. of sodium, sufficient methanol to dissolve and ether to bring volume to 4.2 ml.), which caused separation of a mass of beautiful plates. This material was very hygroscopic, becoming liquid in air after a very short time. The ether was withdrawn by a filter stick and the solid was dried under vacuum to a constant weight of 0.945 g.

After standing 10 weeks at room temperature, sodium phenylacetothiohydroxamate had darkened somewhat and was no longer hygroscopic. The solid was washed with carbon disulfide (two 20-ml. portions) which removed nearly all of the color. The solid partially dissolved in methanol (10 ml.) and the mixture was neutralized with dilute hydrochloric acid. The residue was extracted again with methanol (10 ml.) and the methanol extracts combined. Water (30 ml.) was added and the mixture chilled, yielding 0.4102 g. of crystalline solid, m.p. 128-131°. After two crystallizations from methanol, 0.1680 g. (26%) of N,N'-dibenzyl-thiourea was obtained, m.p. 147.5-148.6°; identified by infrared spectrum, ultraviolet spectrum and melting point of a mixture with a synthetic sample. (Lit. 55)
Preparation of Acetobromoglucose: Acetobromoglucose was prepared from glucose pentaacetate by the method of Scheurer and Smith\textsuperscript{82} or by the following modification:

A brominating reagent was prepared by passing hydrogen bromide gas into acetic anhydride (200 ml.) at 0\(^\circ\) until 208 g. had dissolved. Glucose pentaacetate (50 g.) was added to the cold (ca. 5\(\circ\)) brominating reagent (75 ml.) and the solution was allowed to stand overnight at ca. 5\(\circ\)\(^\circ\). The solution was diluted with chloroform (75 ml.) and poured into ice with rapid stirring. The chloroform layer, containing the acetobromoglucose, was removed and washed successively with ice water (two 50-ml. portions), cold 5\% potassium carbonate solution (25 ml.), ice water (50 ml.) and saturated aqueous sodium chloride (20 ml.). Failure to wash well at this point yields a final product which is difficult to crystallize. After drying over calcium chloride, the chloroform solution was concentrated to a syrup under water pump vacuum with a bath temperature not over 50\(\circ\). The acetobromoglucose was crystallized from ether-hexane; yield 28.1 g., m.p. 84.5-87\(\circ\). A second crop of 4.2 g., m.p. 81-85\(\circ\), was also collected. The total yield was 61\%. Acetobromoglucose decomposed at room temperature but could be stored several months at -5\(\circ\) (Lit. 82)

S-\textsuperscript{Q}-d-l-(Tetraacetylglucopyranosyl)-phenylacetothiohydroximic Acid: This material was obtained in yields from 74\% (impure, m.p. 135-143\(\circ\)) to 47\% (pure, m.p. 163.8-164.1\(\circ\)) by condensing phenylacetothiohydroxamic acid and acetobromoglucose in the presence of sodium methoxide in methanol-acetone solution. One equivalent of acetobromoglucose and sodium methoxide was used with an excess of
thiohydroxamic acid. Only one product was obtained despite efforts to detect another by chromatography on silicic acid. The most satisfactory procedure is described below.

Phenylacetothiohydroxamic acid (5.271 g.) in 10 ml. of acetone was partially neutralized with a 3.1 N solution of potassium hydroxide in methanol (8.75 ml.). Acetobromoglucose (11.34 g.) in 15 ml. of acetone was added causing immediate precipitation of potassium bromide. The mixture was allowed to stir 7 hours and then poured into 300 ml. of water. An oil separated which rapidly solidified and was removed by filtration after 15 minutes. The solid was air dried overnight. The S-\(\beta\)-d-l-(tetraacetylglucopyranosyl)-phenylacetothiohydroximic acid, twice crystallized from water-acetone or chloroform-carbon tetrachloride (as fine needles), melted at 163.8-164.1°, possessed no accessible ultraviolet absorption maximum and was levorotatory, \([\alpha]_D^{22} = -9.62^\circ\) (c = 0.97, chloroform). This material afforded hydroxylamine in 49% yield by acid hydrolysis as described in Section II. (Lit. cf. 40)

Anal. Calcd. for \(C_{22}H_{27}O_{10}NS\): C, 53.11; H, 5.47; N, 2.82; S, 6.45;

Found: C, 53.34; H, 5.66; N, 3.21; S, 6.27.

Attempted Isomerization of S-\(\beta\)-d-l-(Tetraacetylglucopyranosyl)-phenylacetothiohydroximic Acid by Hydrogen Chloride: This material (0.420 g.) was dissolved in 3 ml. of chloroform and 2 ml. of ether was added. The solution was saturated with hydrogen chloride, allowed to stand 5 minutes and taken to dryness. The gum was
dissolved in 10 ml. of chloroform and the solution was washed with 2 ml. of 10% potassium carbonate. The chloroform solution was concentrated to ca. \( \frac{1}{2} \text{ ml.} \) and 4 ml. of carbon tetrachloride added. After standing in the cold crystals formed; 0.0210 g., m.p. 142-158°. This material melted immediately when immersed in a bath preheated to 140° but when mixed with starting material melted at 162-163°. The infrared spectrum (mull) was different from starting material in the 10-13.5 micron region but became identical to that of starting material when the mull was prepared by prolonged grinding. That this was an unstable crystalline modification was demonstrated by seeding a solution of the starting material in chloroform-carbon tetrachloride solution, whereupon the unstable form rapidly crystallized. The unstable form crystallized rapidly in moderately large, thick needles while the stable form crystallized slowly in fine, hair-like needles. The unstable but not the stable form melted in a bath preheated to 140°.

**Sulfonation of S-\( \text{\textbeta}\)-d-1-(Tetraacetylglucopyranosyl)-phenylacetothiohydroximic Acid:** The thiohydroximic acid was sulfonated in pyridine solution by the action of the sulfur trioxide-pyridine complex to furnish the tetraacetylglucotropaeolate ion. By treatment of the sulfonation mixture with a stoichiometric amount of potassium carbonate, sodium carbonate or tetramethylammonium hydroxide solution, the corresponding tetraacetylglucotropaeolate salt was isolated. The sulfur trioxide-pyridine reagent was prepared as described by Fieser and could be stored over phosphorus pentoxide.
at room temperature for several months. The following general sulfonation procedure was used.

One gram of S-(3-d-1-(tetraacetylglucopyranosyl)-phenylacetothiohydroxamic acid was added to a suspension of 1 g. of sulfur trioxide-pyridine reagent in 5 ml. of pyridine. The mixture was allowed to stir at room temperature 12-24 hours.

1. Potassium Tetraacetylglucotropaeolate: Potassium carbonate (ca. 0.9 g.) and 2 to 5 ml. of water were added to the mixture and stirring was continued until gas evolution ceased. Ether (20-30 ml.) was added causing a copious precipitate. The ether was removed by decantation and the solid washed again with a small amount of ether. Crystallization from a small amount of warm water gave tetraacetylglucotropaeolin in 40 to 58% yields, \([\alpha]_D^2 -22.6^\circ\) (water, C:1.4). The melting point of the first preparation (air dried) was ca. 186° d, but subsequent preparations and most of natural origin melted as low as 140-150° d. The infrared spectra (mull) of all samples of synthetic or natural origin were indistinguishable.* X-ray powder patterns of a high melting (ca. 186) and a low melting sample showed no difference. The melting points were determined in capillary tubes in an electrically heated bath without preheating. A synthetic sample, m.p. 139.5-151.0°, had the following analysis.

* Drs. A. Kjaer, R. Gmelin and O. E. Schultz kindly furnished samples of naturally derived potassium tetraacetylglucotropaeolate.
Anal. Calcd. for C$_{22}$H$_{26}$O$_{13}$NS$_2$K-H$_2$O: C, 41.70; H, 4.45;

Found: C, 41.39; H, 4.44.

2. Sodium Tetraacetylglucotropaeolate: This material was obtained in 57% yield by the preceding procedure by substitution of sodium carbonate for potassium carbonate. The melting point of sodium tetraacetylglucotropaeolate, immersed in a bath preheated to 165° and heated 2.5° per minute, was ca. 170° d; [α]$_D^{28}$ -20° (water, C=2.2). A sample was crystallized from methanol–chloroform for analysis; m.p. 171° d.

Anal. Calcd. for C$_{22}$H$_{26}$O$_{13}$NS$_2$Na: C, 44.07; H, 4.37;

Found: C, 44.02; H, 4.22.

Sodium tetraacetylglucotropaeolate prepared above was identical, according to the infrared spectrum, to a sample prepared from the naturally derived potassium salt by use of Amberlite IR-120 resin. The melting point of this material was very dependent on the conditions under which it was determined.

3. Tetramethylammonium tetraacetylglucotropaeolate: To the sulfonation mixture was added aqueous tetramethylammonium hydroxide solution (10%, 9.0 ml.) while cooling in ice. The resulting neutral solution was washed with ether (two 40-ml. portions) and the aqueous phase chilled in ice. A gum separated which was twice recrystallized from ethanol–ether or ethanol–ether–benzene to furnish tetramethylammonium tetraacetylglucotropaeolate in 53% yield; m.p. ca. 180°. A sample, recrystallized from ethanol and dried 48 hours at 2 mm., melted at 182-183.2° without decomposition; [α]$_D^{28}$ -18.9° (water, C=2.5).
Anal. Calcd. for C₂₆H₃₈O₁₃S₂N₂: C, 47.99; H, 5.89;  
Found: C, 47.84; H, 5.95.

This material was hygroscopic, taking up 1 1/4 to 1 1/3 moles of water when equilibrated in air.

Ammonolysis of Tetramethylammonium Tetraacetylglucotropaeolate:  
This material (1.010 g.) was dissolved in dry methanol (50 ml.) which had been saturated with dry ammonia gas at 0°C. The solution was allowed to stand 14 hours at room temperature and then concentrated to dryness in vacuum. The residue crystallized from methanol-ethanol to yield .6782 g. (91%) of tetramethylammonium glucotropaeolate, m.p. ca. 187°C (bath preheated to 180°C); [α]²⁸D -16.7° (water, C=3.4). This material afforded hydroxylamine in 71% yield by acid hydrolysis (see Section II).

Anal. Calcd. for C₁₈H₃₀O₇S₂N₂: C, 44.80; H, 6.27;  
S, 13.29; N, 5.81.  
Found: C, 45.03; H, 6.58;  
S, 13.87; N, 6.08.

Ammonolysis of the potassium or sodium salts did not yield a crystalline product. (Lit. cf. 30)

Ammonolysis of S-β-d-l-(Tetraacetylglucopyranosyl)-phenylaceto- 
thiohydroximide Acid: This material (.5157 g.) was ammonolyzed by the same procedure used above. The S-β-d-l-glucopyranosyl-phenylacetothiohydroximide acid crystallized with difficulty from anhydrous solvents (ethanol with chloroform or benzene) and melted at ca. 120-121°C. Crystallization from ethanol-benzene containing a small amount of water was more rapid and the product melted at ca. 115°C; [α]²⁸D -14.0° (water, C=2.7).
Anal. Calcd. for C_{11}H_{19}O_{6}NS·H_{2}O: C, 48.40; H, 6.09;

Found: C, 48.78, 48.68;
H, 6.09, 6.46.

Hydrolysis of Synthetic Tetramethylammonium Glucotropaeolacte by
Myrosin: The glucoside (.1509 g.) was dissolved in 10 ml. of
phosphate buffer solution (0.1 M potassium dihydrogen phosphate; 0.1 M
disodium hydrogen phosphate). Ether (30 ml.) and myrosin solution
were added and the mixture shaken one hour. The ether phase was re-
moved and diluted to 50 ml. One milliliter of this solution diluted
to 10 ml. with ethanol had a single ultraviolet maximum at 246 m\mu,
d=910.

Myrosin solution (5 ml.) and ether (30 ml.) were again added
to the aqueous phase and the mixture was shaken two hours. Separa-
tion of the ether phase, dilution and determination of the absorp-
tion at 246 m\mu afforded a value of d=135.

To each ether extract and the respective spectral sample was
added concentrated ammonium hydroxide (5 ml.) and the solutions were
allowed to stand overnight at ca. 5. The solutions were assayed
for benzylthiourea by the method of Kjaer and found to contain a
total of $3.2 \times 10^{-4}$ mole (ca. 100%). Evaporation of these solutions
and crystallization of the residue from ethanol-water yielded .0395 g.
(76%) of benzylthiourea, m.p. 162-162.8°, identified by melting point
of a mixture and infrared spectrum with a synthetic sample. (Lit. 83, 84)
S-\(\beta\)-d-L-Glucopyranosylphenylacetothiohydroximic Acid and Myrosin:
The glucoside (.0561 g.) was mixed with 5 ml. of myrosin solution
and 5 ml. of phosphate buffer and allowed to stand at room tempera-
ture twenty-four hours. The solution was evaporated to dryness and the residue crystallized from ethanol-benzene. The recovered 5-S-β-d-L-glucopyranosylphenylacetothiohydroximic acid, .0205 g. (37%), was identified by melting point and infrared spectrum.

Isolation of Tetramethylammonium Glucotropaeolate from Nasturtium Seed: Eighty grams of ground seed was defatted with carbon tetrachloride in a Soxhlet extractor. The residue was boiled 30 minutes in methanol (500 ml.) and then exhaustively extracted in a Soxhlet using the same methanol solution. The methanol solution was taken to dryness, the residue dissolved in water (50 ml.) and cleared by filtration through Celite. This aqueous solution was passed through Amberlite IR-4B resin (3 g.) as described in the isolation of sinigrin. Elution was accomplished with .05 N tetramethylammonium hydroxide solution. Concentration of the effluent and two crystallizations from aqueous ethanol yielded .1721 g. of tetramethylammonium glucotropaeolate, identified by melting point of a mixture and infrared spectrum with the synthetic material.
Bibliography
Bibliography

6. A. Bussy, J. pharm., 26, 39 (1840); Ann., 34, 223 (1840).
14. J. Gadamer, Ber., 32, 2335 (1899); Arch. Pharm., 237, 111 (1899).
15. A. W. Hofmann, Ber., 7, 518 (1874).
17. W. Schneider and L. A. Schutz, Ber., 46, 2634 (1913); 45, 2954 (1912).
20. W. Schneider et. al., Ber., 47, 2218, 1258 (1914).
21. (a) E. Pacsu and E. J. Wilson, Jr., J. Am. Chem. Soc., 61, 1150, 1930 (1939); P. Brigl, K. Gronemeir and A. Schulz, Ber., 72, 1052 (1939). (b) W. Schneider et. al., Ber., 61, 231 (1918); 81, 1257 (1928).


67. O. Wallach and H. Bleibtreu, Ber., 12, 1062 (1879).
70. A. Streitwieser, Jr., Chem. Revs., 56, 518 (1956).
75. H. Wuyts, et. al., Bull. Soc. chim. Belges., 39, 58 (1930); 41, 1926 (1932).
77. A. Hantzsch, Ber., 64, 661 (1931).


83. C. Neuberg and J. Wagner, Biochem. Z., 174, 457 (1926).