AD NUMBER
AD843940

NEW LIMITATION CHANGE

TO
Approved for public release, distribution unlimited

FROM
Distribution authorized to U.S. Gov't. agencies and their contractors; Foreign Government Information; JUL 1968. Other requests shall be referred to the Army Biological Laboratory, Attn: SMUFD-AE-T, Fort Detrick, MD 21701.

AUTHORITY
BDRL, per d/a ltr dtd 13 Sep 1971
This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Commanding Officer, Fort Detrick, ATTN: SMUDP-AE-T, Frederick, Md. 21701.
Best Available Copy
Investigations of mustard oil glycosides. IV. Phenyl-thiourethane-d-glycoside.

by Wilhelm Schneider and Douglas Clibben.


Similarly to the silver salts of other thiourethanes (1), phenyl-thiourethane silver isolated by Liebermann (2) also reacts with acet-o-bromo-glucose, forming a tetra-acetyl-thio-urethane-glycoside corresponding to the equation:

\[ C_2H_5N : C \cdots S(\text{CH}_{2}C")_2O = C_2H_5N : C \cdots S(\text{CH}_{2}C")_2O \cdot 4\text{H}_2\text{O} \]

However, upon saponification of the acetyl groups with barium hydroxide solution, the primary product phenyl-thiourethane-d-glycoside decomposes, under the effect of aqueous alkali, (similarly to the thiourethane-glycosides described previously,) to phenyl-urethane and the decomposition products of thioglucose.

If saponification is effected by means of alcoholic agents, a syrupy reaction product is obtained which, in addition to acetamide, contains only totally undecomposed phenyl-thiourethane-d-glycoside, of which unequivocal proof can be submitted. In spite of numerous attempts, we have not been able to obtain the glycoside in crystalline form, although it is a very stable compound upon complete coagulation of water.

Particular interest is offered by the decomposition to which phenyl-thiourethane-d-glycoside is subjected by the influence of water and acids. It assumes here, as it does in respect to its stability, a median position between thiourethane-glycosides synthesized to date on one hand, and the natural mustard oil glycosides (sinigrin, gluco-cheirolin) on the other. While thiourethane-glycosides, diethanly substituted in connection with nitrogen, decompose and form urethanes and thioglucose, mustard oil glycosides have to date yielded only a cleavage into glucose on one hand, and mustard oil (or its decomposition products) and potassium bisulfite on the other. The hydrolysis of phenyl-thiourethane-d-glycoside progresses in two parallel directions, once likewise with formation of phenylurethane and thioglucose in the sense of equation I, but simultaneously, and similarly to the decomposition of mustard oil glycoside, accompanied by separation of sulfur from the sugar, resulting in the reconstitution of phenyl-thiourethane according to equation II:

\[ C_2H_5N : C \cdots S(\text{CH}_{2}C")_2O = C_2H_5N : C \cdots S(\text{CH}_{2}C")_2O \cdot 4\text{H}_2\text{O} \]

The affinity of synthesized phenyl-thiourethane-d-glycoside to natural mustard oil glycosides is shown with particular clarity by its reaction with silver nitrate. It should be remembered that mustard oil glycosides, upon
treatment with this reagent, yield mustard oil silver sulfates in addition to glucose (3), a process formulated by Gadamer in the following manner:

$$R \cdot N \cdot C \left( \frac{S \cdot C \cdot H \cdot O}{O \cdot C \cdot H \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \·
150°C, corresponding to 62% of the theory.

The pure compound, recrystallized from hot alcohol, melts sharply at
150°C (uncorrected). It is insoluble in water, also in cold ligroin, benzene,
toluene, xylene and alcohol, but dissolves easily in other, chloroform and in
hot alcohol and xylene. Reactions with alkaline lead solution and rechling's
solution are the same as those of other acetyl-thiothiourethane-glycosides (5)
described heretofore.

\[
\text{C}_8\text{H}_8\text{O}_4\text{NS} \quad \text{Calculated: C 52.65, H 5.72, S 8.97}
\]
\[
\text{Found: C 52.60, H 5.52, S 8.49}
\]

The optical determination was made with the solution in acetylene tetra-
chloride.

For sodium light, alpha at 15°C equals -0.22° (2 cm tube, concentration
4.4650).

Therefore \( \alpha = -0.22° \).

Saponification of
tetraacetyl-phenyl-thiourea-thioglycoside.

a) With baryta water: The effect of baryta water alone on tetraacetyl-
glycoside is extraordinarily slow; it is speeded up somewhat by the addition
of a little alcohol to the suspension of the acetyl-glycoside. 5 g of acetyl
compound were shaken for 2 days with a solution of 10 g crystallized barium
hydroxide in 200 cm water and 100 cm alcohol. The resulting clear solution
was separated from excess baryta by means of carboxic acid and concentrated
under reduced pressure at 30°C. After removal of alcohol by distillation, a
brown oil, soluble in ether, was obtained. The ethereal solution, after
evaporation, yielded 0.7 g of a dark oil, which set in crystalline form after
standing for several days. The crystals were recrystallized from hot boiling
ether. They were free of sulfur and melted sharply at 52°C (uncorrected),
therefore consisted of phenyl-urethane. The aqueous mother liquor of saponi-
fication, extracted with ether, developed hydrogen sulfide on acidification,
which evidently had formed by decomposition of primary thioglucoside with alkali.

b) With alcoholic ammonia. 5 g acetyl-glycoside were dissolved in 400 cm
hot absolute alcohol. The solution, after cooling to 0°C, was saturated with
dry ammonia gas under occlusion of moisture. The acetyl-glycoside which had
initially separated in fine-crystalline form during cooling, now dissolved in
a clear solution within a few hours, aided by occasional agitation. The solu-
tion now rested for 2 days at room temperature, then was free of dissolved
ammonia at the vacuum pump. Subsequent to evaporation of the alcohol under
low pressure, a syrupy residue was obtained, which could not be transformed
into a crystalline product by any method. The non-alcoholic syrup is insoluble
in ether, but is very easily dissolved with a little alcohol. In this concentrated alcoholic solution, however, even a large admixture of ether cannot cause significant separation. The syrup dissolves easily in water, and quite clearly, if it is freshly produced or stored under strict exclusion of humidity. This shows that the syrup contains, in addition to acetamide, only totally undecomposed phenyl-thiourethane-d-glycoside, since otherwise water-insoluble decomposition products would be visible. The syrup proved to be dextrorotatory in the polarization apparatus.

**Hydrolysis of phenyl-thiourethane-d-glycoside.**

The clear aqueous solution of the syrupy acetamide-glycoside mixture gradually becomes turbid upon standing at room temperature, and deposits an oily emulsion which settles after a few hours. After a longer period of standing, small amounts of phenyl-thiourethane with a melting point of 72°C (uncorrected) crystallize from the aqueous solution above the oil. As the following test will prove, the oil consists of phenyl-urethane, whose crystallization is prevented by the admixture of a little thiourethane.

The syrupy saponification product from 5 g tetraacetyl-phenylthiourethane-d-glycoside was dissolved in 100 ccm water and allowed to stand at room temperature for 2 days. The oily deposit was absorbed in ether together with the crystalline sediment. After evaporation, the ether yielded about 1.5 g of an oily residue that was dissolved in about 100 ccm of 80% alcohol. By the addition of a sufficient amount of ammoniacal silver solution, phenyl-thiourethane was separated as silver salt which gradually crystallized in the form of needles. It melted at 206°C (uncorrected) and amounted to 0.3 g, corresponding to about 0.16 g phenylthiourethane. The alcoholic mother liquor was freed of alcoholic content under reduced pressure, the residue was absorbed with ether, dried, and isolated by separation from ether. Thus about 1.3 g of an oil were obtained which rapidly solidified in crystalline form after inoculation with a crystal of phenylurethane. The crystalline mass was recrystallized from petroleum ether and the compound identified by its melting point as phenyl-urethane. In the original aqueous glycoside solution, freed with ether of urethane and thiourethane, the presence of thioglucose as the cleavage product corresponding to phenyl-urethane could be proved by the addition of ammoniacal silver solution and much alcohol, the silver salt of which was separated in this manner in the characteristic, amorphous, yellow, easily water-soluble flakes.

Upon boiling with diluted acids the hydrolysis of phenyl-thiourethane-d-glycoside progresses similarly, but much faster. However, phenyl-thiourethane seems to be produced in preponderant amounts, since the obtained water-insoluble oil solidified rapidly into crystals during cooling, which melted directly at 70-71°C. The direction in which phenyl-thiourethane-d-glycoside is predominantly split during hydrolysis, apparently depends on the reaction of its aqueous solution. In alkaline solution (see baryta saponification) only phenylurethane was observed as sugar-free cleavage product. In aqueous solution urethane and thiourethane are obtained simultaneously; in acid solution, primarily phenyl-thiourethane (6).
The enzyme myrosin, which splits mustard oil glycosides, significantly has no noticeable effect on phenyl-thiourethane-α-glycoside. In two parallel tests, in which equal quantities of glycoside were subjected to aqueous hydrolysis under otherwise identical conditions, once without, once with admixture of myrosin solution, the amounts of oily decomposition products extracted from time to time with ether, were quantitatively equal.

**Reaction of phenyl-thiourethane-α-glycoside with silver nitrate.**

The saponification product of tetraacetyl-phenyl-thiourethane-α-glycoside was dissolved in 100 cc of 80% alcohol and mixed with 10 cc silver nitrate solution (1:10). After a short time thioglucoce silver separated in the form of an amorphous, brown powder. The solution was stored in the refrigerator for 4 hours to complete the reaction. Subsequently the separated powder was drawn off by suction through a layer of siliceous earth and decomposed by introduction of hydrogen sulfide. After filtration from the silver sulfide precipitate, a colorless, aqueous solution was obtained, which was completely freed of dissolved hydrogen sulfide at the vacuum pump. After the addition of 3 cc of an ammonical silver nitrate solution (1:10), thioglucoce silver separated in purified form. It formed snow-white, amorphous flakes which during sedimentation turned slightly yellowish. The remaining liquid was poured off, the precipitate was again decanted with 100 cc 80% alcohol and finally drawn off by suction. The preparation was first dried for one day in vacuo over phosphorous pentoxide, then to constant weight at the temperature of chloroform vapor. The yield amounted to 0.65 g. The silver-thioglucoce thus obtained represented a weakly yellowish, amorphous, very stable powder, which even withstood extended heating at 100°C (water steam) without apparent change. When heated in the melting point apparatus, it gradually turned brown at above 100°C and melted at about 165°C after turning black. It could be demonstrated that the increased stability of this preparation is compared to previously described ones (7) is attributable to the circumstance that an excess of silver solution had been avoided in the precipitation of thioglucoce; on the contrary, too little silver had been used. Even in aqueous solution this preparation was quite stable, it even withstood short boiling, whereas, more intensive yellowing only developed very slowly. However, a trace of excessive silver salt solution caused rapid decomposition even at room temperature, accompanied by blackening, of the aqueous solution of thioglucoce-silver, as well as of the flaky suspension freshly precipitated therefrom with alcohol. The preparation was entirely free of nitrogen; nevertheless the silver content was found to be about 26 too low, similarly to previous experience, while the sulfur content agreed well with the formula for thioglucoce-silver. This result probably can be explained most simply by saying that the silver compound has adsorbed and carried over a small quantity of thioglucoce (or some disulfide resulting therefrom).

O. C. + H. O. = 0. C. H. + 0. S. 8. Ag. Calculated: S 18.58, N 35.5%. Found: S 10.55, N 33.45%
The original, silver nitrate-containing reaction mixture, filtered off from crude thioglucose silver, was mixed with an excess of ammonia. Copious quantities of phenyl-thiourethane silver crystals separated out, which were identified by their solubility and melting point. Hence it follows that, in the presence of silver nitrate, the decomposition of phenyl-thiourethane-d-glycoside takes place in both of two possible directions.

NOTES

(2) Paper 207, 142 (1881).
(3) Gadamer, paper 235, 47 and following (1897); W. Schneider and E. A. Schultz, Vol. 46, 2633 (1913).
(4) Paper 207, 142 (1881).
(5) Vol. 47, 1261 and following (1914).
(6) To a certain degree, aminogin reacts similarly. Silver nitrate in aqueous solution (i.e., in a solution which rapidly turns acid owing to freed nitric acid) produces from it only allyl-mustard oil silver sulfate and glucose. Through the effect of alcoholic ammonia, a small amount of thioglucose results as the decomposition product of this natural mustard oil glycoside. Cf. the following paper.
(7) See Vol. 47, 1263, 1265, 1268 (1914).