

***iso*Thiocyanates VIII. Synthesis of *p*-Hydroxybenzyl
*iso*Thiocyanate and Demonstration of its Presence in the
Glucoside of White Mustard (*Sinapis alba* L.)**

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Sinalbin, the *isothiocyanate* glucoside in seed of white mustard (*Sinapis alba* L.), is enzymatically hydrolyzed to glucose, the sulphate of the base sinapine and a non-volatile *isothiocyanate*. The latter has never been isolated in the pure state or adequately identified. Will and Laubenheimer¹ obtained upon treatment of the silver salt of sinalbin with hydrogen sulphide a nitrile, which on acid hydrolysis yielded what they believed to be *o*-hydroxyphenylacetic acid. Salkowski² later revised this structure to the corresponding *para*-isomeride and accordingly formulated the mustard oil of sinalbin as *p*-hydroxybenzyl *isothiocyanate* (I). His attempts to substantiate this assumption by a synthesis of (I) resulted in the production of an oily, vesicant substance for which neither analyses nor other proper characterisation were presented. The extraordinary lability of (I) may explain this as well as more recent failures to synthesise and characterise the *isothiocyanate* of white mustard seed, as pointed out in the review of André³. The fact that seed of *Sinapis alba* constitutes an important ingredient in the manufacture of table mustard seemed to us to warrant a reëxamination of the *isothiocyanate* contained therein.

A few preliminary synthetic experiments sufficed to indicate the great difficulties attending the preparation of (I) in analytically pure form, due to oxidation, polymerization and maybe other secondary reactions. Therefore, attention was turned to the possibility of removing the desired *isothiocyanate* immediately after its formation by means of an amine, thereby transforming the labile compound into an equally characteristic but more tractable thiourea-derivative. Eventually, this technique proved successful although a great many experiments were needed in order to establish the rather special conditions required for the reaction to proceed as desired.

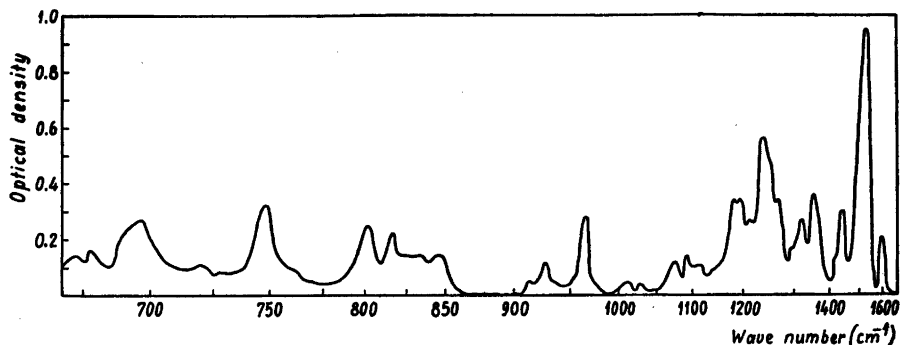
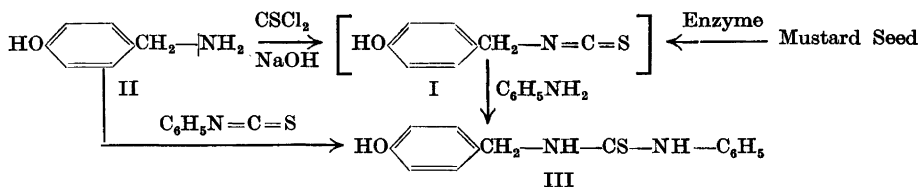


Fig. 1. Infra-red absorption spectrum of *N*-*p*-hydroxybenzyl-*N'*-phenyl-thiourea (III) dispersed in a potassium bromide plate.

p-Hydroxybenzylamine, needed as a starting material, was prepared by catalytic reduction of *p*-hydroxybenzaloxime, essentially as described by Ott and Zimmermann⁴. Various methods were investigated for the transformation of the amine (II) into the corresponding isothiocyanate (I). These included the Hofmann synthesis, the modified Andreasch-Kaluza procedure (*cf.* Ref. ⁵) as well as a great number of modifications of Dyson's method^{6,7}, employing thiocarbonyl chloride. Most attempts proved completely abortive and resulted in intractable, dark and gummy products which showed no signs of yielding crystalline derivatives upon treatment with a rather wide selection of amines. Intermolecular condensations, in which both the isothiocyanate- and hydroxy-grouping are involved, seem to be mainly responsible for the formation of secondary products, mostly of polymeric nature. It was eventually found, however, that the reaction between (II) and thiocarbonyl chloride, when conducted under special circumstances as described in the experimental part below, proceeded in the desired way to give (I). Its presence in the solution appeared from the isolation of a crystalline thiourea-derivative (III) upon reaction with aniline.



To support the structure (III) another specimen was independently prepared from the amine (II) and phenyl isothiocyanate. Finally, the connection to the natural product was made when it was observed that the isothiocyanate, liberated upon enzymatic hydrolysis of defatted, pulverised seed of white mustard, could be continuously removed by ether extraction and directly transformed into a phenylthiourea-derivative on reaction with aniline.

After careful purification the three, independently prepared derivatives proved identical, having the same melting point undepressed on mutual admixture. Further proof of the identity was furnished through determination of the infra-red absorption spectra of the three specimens of N-*p*-hydroxybenzyl-N'-phenyl-thiourea (III). The identical infra-red spectrum of the three thioureas is presented in Fig. 1. The very intensive line at $1\,532\text{ cm}^{-1}$ is attributable to the C=S-grouping, while the band exhibited at $1\,437\text{ cm}^{-1}$ is due to the benzene rings. A rather strong absorption at $1\,437\text{ cm}^{-1}$ may be assigned to the CH₂-deformation vibration while the bands at 748 cm^{-1} and $800\text{--}815\text{ cm}^{-1}$ probably arise from the mono- and *p*-disubstituted benzene rings, respectively.

Thus it has been definitely proved that *p*-hydroxybenzyl isothiocyanate (I) is the mustard oil liberated on enzymatic hydrolysis of seed of white mustard as suggested many years ago by Salkowski², although its extraordinary labile character makes it rather doubtful that an analytically pure sample could be prepared.

EXPERIMENTAL *

p-Hydroxybenzaldoxime. The following procedure was found more convenient than the one previously reported⁴. To a suspension of 95 g of *p*-hydroxybenzaldehyde and 81.5 g of hydroxylamine hydrochloride in 500 ml of water was added a solution of 40.5 g of sodium hydroxide in 320 ml of water and enough alcohol to give a clear solution. This was boiled under reflux for one hour, the main part of the ethanol removed *in vacuo* and the aqueous phase successively extracted with one 200 ml and three 100 ml portions of ether. The ethereal phase was dried over sodium sulphate and the ether removed whereupon the oxime separated. It recrystallised from a large volume (6 l) of benzene as beautiful, slightly pink crystals (80 g, 75%), m.p. $115\text{--}116^\circ$. (Found: N 10.18. Calc. for C₇H₇O₂N; N 10.22).

From the mother liquor an additional crop (15 g) could be obtained which melted at $112\text{--}113^\circ$. Ref.⁴ reports the m.p. 112° for the anhydrous oxime.

p-Hydroxybenzylamine (II). The oxime was catalytically reduced by a modification of the procedure of Ott and Zimmermann⁴.

To a solution of 15 g of the oxime in 750 ml of 96 % ethanol were added 300–400 mg of Adam's PdO-catalyst and 10 ml of concentrated hydrochloric acid. The mixture was shaken at ordinary temperature and pressure in a hydrogen atmosphere. Within 6–7 hours a total amount of 6.6–6.7 l of hydrogen had been absorbed whereupon the uptake suddenly stopped. The catalyst was removed by filtration through a layer of Celite and the filtrate concentrated to a small volume *in vacuo*. Upon addition of ether, *p*-hydroxybenzylamine hydrochloride separated as a colourless, crystalline powder (12.9 g, 74 %), m.p. $190\text{--}191^\circ$. (Found: Cl 22.34. Calc. for C₇H₁₀ONCl; Cl 22.21). Ref.⁴ reports the m.p. 195° for the hydrochloride.

p-Hydroxybenzyl isothiocyanate (I) (in solution). After many experiments the following procedure was eventually adopted as the most suitable for the preparation of a dilute ether solution of (I). A three-necked flask, to the bottom of which was sealed a wide-bore stopcock, was equipped with a separatory funnel, in- and outlets for a nitrogen stream and an effective, mercury-sealed stirrer. In the flask were placed 1.08 g of thiocarbonyl chloride dissolved in 25 ml of ether and 1.5 g of *p*-hydroxybenzylamine hydrochloride in 20 ml of water. The apparatus was flushed with nitrogen and a slight nitrogen pressure was kept in the system throughout the following operations.

* All melting points are uncorrected and determined in capillary tubes in an electrically heated block.

The flask was now immersed in an ice-bath and a solution of 1.12 g of sodium hydroxide in 10 ml of water added from the separatory funnel under vigorous stirring in the course of 10 minutes. After stirring for another 10 minutes, the lower phase was drained off through the stopcock whereas the desired isothiocyanate remained in the ether phase as shown by the following reaction.

N-p-Hydroxybenzyl-N'-phenyl-thiourea (III) from *p-hydroxybenzyl isothiocyanate*. To the ether phase above was dropwise added from the separatory funnel a solution of 0.72 g of aniline in 5 ml of ether. Stirring was continued at room temperature for 24 hours whereupon the clear solution was concentrated *in vacuo* at ordinary temperature. A crystalline mass embedded in a brownish oil remained. The latter was carefully removed with a little ethyl acetate and the crystalline residue was recrystallised several times from dilute ethanol and finally once from aqueous *n*-propanol. The colourless crystals (40 mg) melted at 166°, undepressed on admixture with the independently synthesised specimens reported below. (Found: N 10.92; S 12.31. Calc. for $C_{14}H_{14}ON_2S$ (258.3): N 10.85; S 12.41).

(III) from *p-hydroxybenzylamine*. To a concentrated solution of 0.53 g of *p*-hydroxybenzylamine in ethanol was added 0.64 g of phenyl isothiocyanate and the mixture was refluxed for half an hour. Upon cooling and addition of water an oil separated which soon solidified in the ice-box. Beautiful colourless needles separated on recrystallisation from aqueous ethanol, m.p. 167–168° alone or in admixtures with the two other preparations. (Found: N 10.83; S 12.40).

(III) from seed of *Sinapis alba*. White mustard seed was thoroughly ground in a mill and the powder exhaustively defatted with hot ether. Fifty-seven grams of the resulting material were shaken with 400 ml of water for four hours. The suspension was separated in a high-speed centrifuge and the supernatant completely cleared upon filtration through Celite. It was next placed in a liquid-liquid extractor and continuously extracted with ether for 48 hours. Before starting the extraction, 0.26 g of aniline was placed in the flask containing the ether to be cyclised, thereby securing immediate reaction between the amine and the labile isothiocyanate carried over by the solvent.

Upon concentration of the ether phase a crystalline material separated in a brownish oil which could be removed by careful treatment with ethyl acetate. Repeated recrystallisations from aqueous ethanol afforded the thiourea as beautiful colourless needles, m.p. 167–168°, undepressed again upon admixtures with the samples above. (Found: N 10.82; S 12.21).

Infra-red absorption spectra. The infra-red spectra of the three thiourea samples were determined in potassium bromide plates on a Beckman IR 2 instrument.

SUMMARY

p-Hydroxybenzyl isothiocyanate has been synthesised in ether solution from *p*-hydroxybenzylamine and thiocarbonyl chloride. Its identity has been established upon comparison of its phenylthiourea-derivative with an authentic specimen of *N-p*-hydroxybenzyl-*N'*-phenyl-thiourea, prepared from *p*-hydroxybenzylamine and phenyl isothiocyanate.

The isothiocyanate formed during enzymatic hydrolysis of the glucoside of white mustard (*Sinapis alba* L.) has been definitely proved to be *p*-hydroxybenzyl isothiocyanate through its reaction with aniline, giving a thiourea identical with the two independently prepared derivatives above.

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