

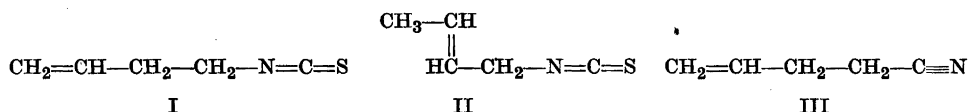
isoThiocyanates III. The Volatile isoThiocyanates in Seeds of Rape (*Brassica napus* L.)

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Although the essential mustard oil in seeds of rape (*Brassica napus* L.) has been repeatedly investigated, no clarity exists as to its chemical nature. Considerable practical interest is attached to this problem, because the *iso*-thiocyanate contents have been made responsible for the toxic manifestations occasionally observed upon feeding rape seed cakes in larger quantities to animals.

In 1899 Jørgensen¹ attributed the toxicity of impure rape seed cakes to the presence of a C₅- (or C₆-) *isothiocyanate* besides the allyl derivative. At about the same time Sjollem² reported the isolation of a mustard oil to which the structure (I) was ascribed, mainly on basis of refraction measurements. These, however, seem to be of questionable value as a structure proof.



Stein³ obtained from Indian rape seed cakes a C₅-*isothiocyanate* which he regarded as the *trans*-crotyl derivative (II). Several years later, Schmalfuss *et al.*⁴ reported the isolation of “crotonylcyanid” (III), “crotonylsenföl” (I) and a higher-boiling *isothiocyanate* of unknown structure upon fractionation of the crude rape seed mustard oil. Neither analyses nor other chemical evidence were presented in support of the suggested structures. André and Kogane-Charles⁵ described the isolation from rape seeds of an *isothiocyanate* for which the structure (II) was suggested, though convincing experimental support was missing. André and Delaveau⁶ recently presented evidence for the presence of three individual volatile *isothiocyanates* in rape seeds. Again, no suggestions as to their chemical nature were made.

When defatted rape seeds were submitted to our routine method of investigation, described in the following communication⁷, a paper chromatogram resulted (Fig. 1) which left no doubt as to the presence of three individual volatile *isothiocyanates*. The major component, the thiourea of which gives

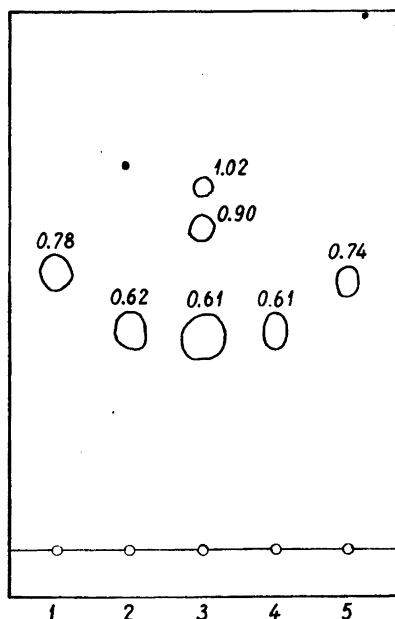


Fig. 1. Paper chromatogram showing the location of: 1. Crotylthiourea; 2. β -Methallylthiourea; 3. The thiourea mixture obtained from rape seed distillates; 4. 3-Butenylthiourea; 5. α -Methallylthiourea. The numbers indicate the R_{Fh} -values (cf. Ref.⁹). Solvent system: chloroform-water.

rise to a spot with an R_{Fh} -value of 0.61, undoubtedly represents the C_5 -isothiocyanate discussed in the literature quoted above. It appears from the paper chromatogram (Fig. 1) that of the four synthetic isomerides previously described⁸, the α -methallyl and *trans*-crotyl derivatives can be definitely ruled out as constituents of rape seeds. The chromatographic method does not allow, however, to discern between 3-butenyl- and β -methallylthiourea, although the previously reported melting points²⁻⁶ of the thiourea-derivative of the isothiocyanate from rape seeds, point to the latter as being the 3-butenyl derivative. This assumption, however, required confirmation by isolation of the major volatile component, transformation into its thiourea derivative and comparison of the latter with the synthetic isomerides on hand.

Preliminary attempts to provide a homogenous sample upon recrystallization of the crude, semi-crystalline thiourea fraction proved unavailing. Neither adsorption chromatography on activated alumina nor the application of starch and cellulose powder partition columns under varying conditions yielded promising results. Lastly recourse was taken to a counter-current distribution of the crude thiourea fraction between chloroform and water. As the result of a paperchromatographic comparison with a large number of synthetic model thioureas⁹, the three components in the rape seed distillates were tentatively regarded as 3-butenyl-, benzyl- and β -phenylethyl thiourea, respectively. On this assumption the distribution coefficients of these compounds in the system chloroform-water were spectrophotometrically determined at 23°. From the values, 0.49, 2.41 and 5.88 respectively, a theoretical distribution pattern (Fig. 2) could be calculated. It appeared that in a twelve-plate procedure the vessels with the serial numbers 0—4 should contain essentially pure

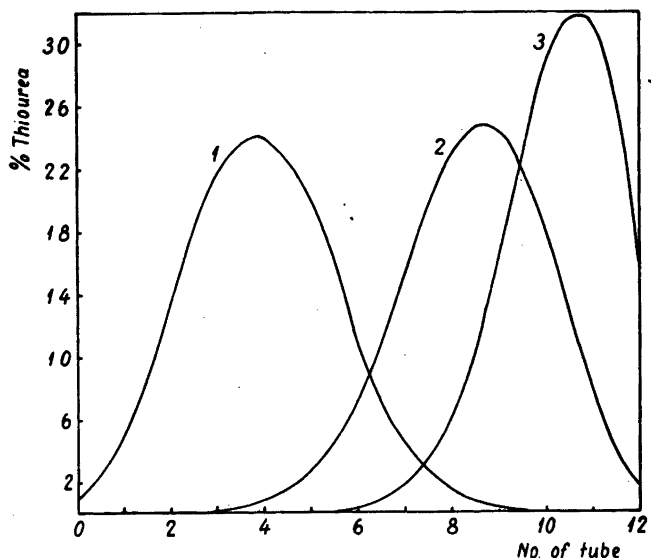


Fig. 2. Hypothetical distribution pattern of: 1. 3-Butenylthiourea; 2. Benzylthiourea; 3. β -Phenylethylthiourea. Solvent pair: chloroform-water.

material of the major component, provided equal amounts of the two phases were employed. The experiment was carried out in twelve separatory funnels and the contents of each funnel checked by paper chromatography. In accord with the theoretical predictions, the first five funnels proved to contain solely the major constituent, whereas no complete separation of the remaining two thioureas could be achieved by this procedure. Upon evaporation of the pooled contents of the five vessels a crystalline product, melting at $65.5\text{--}66.0^\circ$, separated. Analyses confirmed the composition $\text{C}_5\text{H}_7\text{NS}$; mixed melting point determination and infrared spectra (Fig. 3) served to establish its identity with an authentic specimen of 3-butenylthiourea, prepared as described in a preceeding paper of this series⁸. Thus it has been definitely proved that 3-butenyl isothiocyanate (I) is the main constituent of the volatile isothiocyanate fraction from rape seeds.

By spectrophotometrical evaluation of the contents of the separatory funnels Nos. 0—4, the total amount of 3-butenyl isothiocyanate was found to be *ca.* 100 mg per 100 g of defatted rape seeds. This amount proved rather independent of the provenience of the seeds; summer and winter seed species were found about equally rich in isothiocyanates. On the other hand, industrially produced rape seed cakes showed strikingly great variations in their contents, apparently dependent on the conditions employed during the manufacture. This problem is being further studied at present and the results will appear in a forthcoming paper.

The occurrence of 3-butenyl isothiocyanate is not confined to rape seeds. Other *Brassica* members¹⁰ as well as seeds of various other *Cruciferae*⁷ have

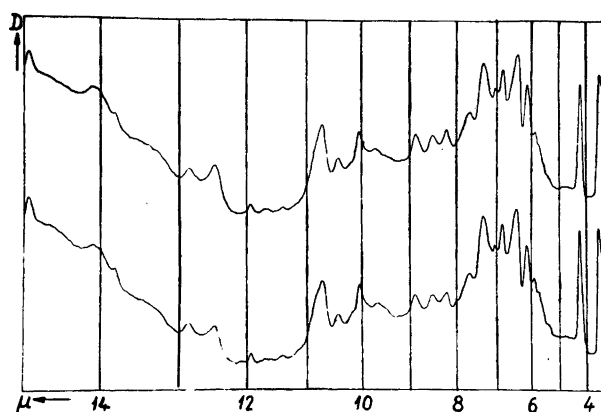


Fig. 3. Infra-red absorption spectra determined in Nujol mulls. Upper curve: synthetic 3-butenylthiourea. Lower curve: thiourea, derived from the major isothiocyanate constituent of rape seeds.

been demonstrated by paper chromatography to contain the 3-butenyl derivative, most often in combination with other isothiocyanates.

The nature of the two minor components of rape seeds is still unknown. Partition chromatography of the middle fractions (Nos. 5—7) from the counter-current distribution on kieselguhr columns afforded a paperchromatographic homogenous thiourea fraction with an R_{Fk} -value of 0.90. The product did not crystallise well and more material is needed in order to reveal its chemical nature. Contrary to expectations, preliminary studies have indicated that the substance is not benzylthiourea, but rather a derivative of a purely aliphatic C_6 - or C_7 -isothiocyanate. The identification of the latter as well as the third constituent, which is present in rape seeds in only very small amounts, is still being prosecuted.

EXPERIMENTAL

Isolation of a crude thiourea fraction from rape seeds. Industrially produced rape seed cakes, containing less than 1 % of fatty material, were used as a convenient starting material for the isolation and further investigation of the volatile isothiocyanates in rape seeds. In cases, where untreated seeds were investigated, a rapid denaturation of myrosinase by a hot ligroin-ethanol mixture followed by exhaustive extraction with petroleum ether, was the standard pretreatment employed.

Five hundred grams of finely divided rape seed cakes were suspended in 3 l of distilled water. To the mixture were added 26 ml of a cell-free myrosinase preparation, made according to Neuberg and Wagner¹¹. After standing at room temperature for 20 hours in a tightly stoppered flask, the liberated isothiocyanates were removed in a stream of steam. The extensive foaming could be somewhat diminished on adding a small amount of ethanol before the distillation. The distillate (10 l) was collected in an ice-cooled receiver containing 4 l of concentrated aqueous ammonia. The clear solution was left overnight at room temperature and then concentrated to dryness *in vacuo* at a bath temperature not exceeding 55°. The semi-crystalline residue (1.80 g) was dissolved in 100 ml of water-saturated chloroform and a drop of the solution used for paper chromatography, the

result of which is shown in Fig. 1. An additional amount of 200 ml of water-saturated chloroform was now added and the solution submitted to the countercurrent separation procedure.

Fractionation of the crude thiourea mixture. In each of 13 separatory funnels (1 liter) were placed 300 ml of water, saturated with chloroform. In the first funnel (No. 0) the chloroform solution of the thiourea mixture was introduced and the contents shaken for about 2 minutes. The chloroform phase was then transferred to the next funnel (No. 1) and fresh chloroform introduced in separatory funnel No. 0. The process was repeated until the first chloroform phase had reached the last vessel (No. 12).

The contents of the separatory funnels were individually evaporated *in vacuo* to a volume of 100 ml at a bath temperature not exceeding 40°. By this procedure the chloroform was completely removed and the individual aqueous solutions were spectrophotometrically evaluated for their thiourea contents, as described in a following paper⁷. After further concentration of the solutions to dryness, methanolic solutions of about 1 % concentration were prepared and utilised for paper chromatography. This revealed that material from the separatory funnels Nos. 0–5 consisted of essentially homogenous material of the predominating thiourea constituent, possessing an *R_{Ph}*-value of 0.61. Therefore, these fractions were combined and carefully recrystallized from aqueous methanol. An additional recrystallization from water yielded 163 mg of beautiful colourless needles, m.p. 65.5–66° (uncorr.), alone or in admixture with an authentic specimen, prepared as previously described⁸.

C ₆ H ₁₀ N ₂ S (130.2)	Calc.	C 46.13	H 7.75	N 21.53	S 24.64
	Found	» 46.39	» 7.84	» 21.72	» 24.53

The infrared spectra (Fig. 3) further served to ascertain the identity of the thiourea.

Absorption spectra. The ultra-violet absorption measurements were performed with a Beckman model DU quartz spectrophotometer. The infra-red spectra were determined in Nujol mulls with a Beckman IR 2 instrument.

SUMMARY

By paper chromatography, the presence of three volatile isothiocyanates in enzymatically treated rape seeds has been demonstrated.

The main constituent has been definitely proved to be 3-butenyl isothiocyanate.

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