The optically active amines were transformed into optically active isothiocyanates as described in the literature [8,12,13] except that (*R*)-menthylisothiocyanate was prepared by the thiophosgen method.

The thiosemicarbazides were prepared as described by Jensen *et al.*[14] for the racemic thiosemicarbazides from the optically active isothiocyanates and hydrazine or substituted hydrazines. With the exception of (*S*)-4-α-methylbenzylthiosemicarbazide ((*S*)-4-α-phenethylthiosemicarbazide) which has been described by Ohlsson [13] all the optically active thiosemicarbazides are new. For analyses, *etc.*, see Table 2.

The thiosemicarbazide complexes were prepared from nickel(II) chloride and the thiosemicarbazides in alcoholic solution as described by Jensen and Rancke Madsen.[2] Usually the complexes were not isolated but the alcoholic solutions were used directly for the measurements (see Table 1).

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Studies on the Determination of Isothiocyanates and Vinyl-Oxazolidinethione in Seeds of Rape and Turnip Rape

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As the amount of thioglucosides in seed meals from rape and turnip rape limits their use as animal feed, investigations into the varietal differences in content of the split products from the thioglucosides were initiated.[1,2] It was found, however, that the analytical methods of Wetter [3,4] were not wholly satisfactory, with regard to the precision obtainable. Furthermore a less tedious method was regarded as essential.

The following steps of the procedure of Wetter have been studied and changed, *viz:* the pH of the myrosinase reaction, the separation of isothiocyanates (I) from oxazolidinethiones (II), and the determination of I. Also pretreatments of the seed and the defatted seed meal are included in our method. According to Schwimmer [5] the pH-optimum of the myrosinase reaction is 6—7. Wetter,[3] however, reported the optimum conditions for release of I from rapeseed meal to be at pH 4. This discrepancy prompted us to look for some destructive agents in crude rapeseed meal.

Van Etten *et al.*[6] found higher II content in *Crambe Abyssinica* seed meal, when hot water extracts of thioglucosides were incubated at pH 7, than at pH 4 as in Wetter's method. They estimate, however, volatile I after release at pH 4. As I at pH > 5 reacts with proteins (see, *e.g.*, Edman [7] and Goksöyr [8]), an apparent pH-optimum of 4 for the I release, as reported by Wetter, is easily explained.

André [9] avoided the loss of I in unbuffered reaction systems by treating the meal with hot water, cooling the slurry and adding a myrosinase preparation.

Attempts to extract the thioglucosides by successive hot water aliquots as in the procedure of van Etten *et al.*[6] were found to give higher II values than when the slurry was incubated at pH 4. This is partly due to the slow cyclization of the 2-hydroxy-3-butenylisothiocyanate to vinyloxazolidinethione at acid pH. As the

process, besides being tedious, gave a lower yield of I and a fairly low precision with our material, further improvements were necessary. To prevent the loss of I when adding the hot water to the meal, the intact seeds were heated to 90°C in a closed vessel for 15 min. This process inactivates the myrosinase in the seed and prevents the release of I whilst adding the water to the meal. Further the repeated extraction with hot water was found to be unnecessary as a simple hot water treatment was equally effective in preventing isothiocyanate-protein reactions.

Selective extraction of I from the digest was thought to be a more rapid and a more exact method of separating the two groups of compounds than by steam distillation.

2,2,4-Trimethyl-pentane with negligible UV-absorption was found to extract I quantitatively, leaving II in the aqueous solution. As the UV-absorption of the thiourea derivatives is about 10 times greater than that of I at the absorption maximum, a conversion of the latter to tne former was required. This was accomplished by a reaction of the 2,2,4-trimethyl-pentane solution with 0.9 N NH_3 in absolute ethanol.

Two different seed samples have been analysed according to Wetter [3,4] and according to the new method.

Table 1 shows the mean of 4 single determinations of the two seed samples used. The considerably higher II values obtained at pH 7 are obvious when comparing the two methods. The new method also gives higher I values on our material than in Wetter's method, mostly since the inherent losses in the steam distillation process are avoided.

Total thioglucoside content calculated from the sum of I and II values, determined according to our procedure, agrees well with the content calculated from determinations of enzymatically released sulphate. The precision obtained with this new method is considerably better, than what has generally been obtained with the older method.

The new method was used as follows:

After heating the air dry seeds at 90°C for 15 min, in a closed vessel, they are defatted with hexane. 1.0 g of the defatted meal is placed in a 250 ml Erlenmeyer flask to which is added 100 ml of boiling 0.2 M phosphate buffer at pH 7. The flask is kept in boiling water for some minutes and then cooled to below 30°C. 3 ml of a 0.5 % aqueous solution of a myrosinase preparation is added. The flask is tightly stoppered and shaken for 3 h and 45 min. 50 ml 95 % ethanol is added and the flask is shaken for another 15 min. Then the mixture is filtered and two samples of 2 ml each are taken from the first 20 ml. The I are extracted from the digest with three portions of 8 ml of 2,2,4-trimethyl-pentane. The combined extracts are made up to 25 ml with the solvent. This solution is

Table 1.

Method	Winter rape var. Matador				Summer turnip rape var. Bele			
	Isothiocyanates mg/g dry meal		Vinyl-oxazolidi-nethione mg/g dry meal		Isothiocyanates mg/g dry meal		Vinyl-oxazolidi-nethione mg/g dry meal	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
No pretreatment, pH 4, steam distillation	4.2	0.2	8.3	0.2	4.8	0.1	1.9	0.1
Pretreatments as in text, pH 7, selective extraction	5.3	0.1	13.1	0.2	5.7	0.1	3.4	0.1

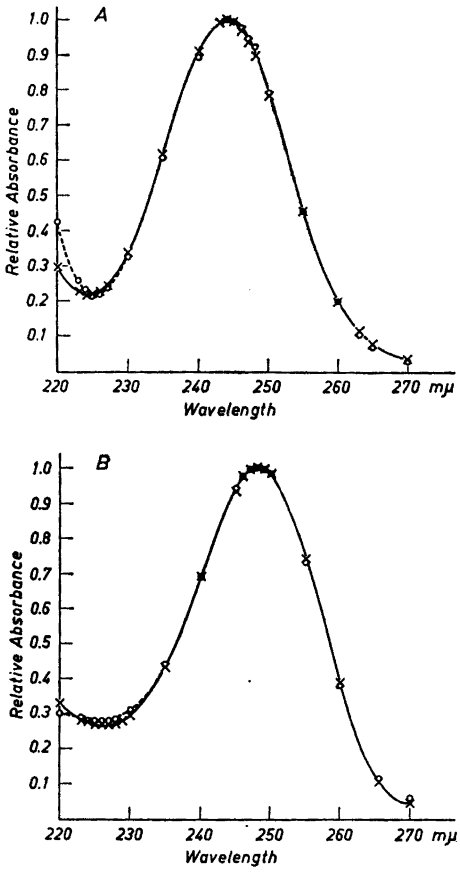

Fig. 1. A: The UV-spectra of thioureas from a pure preparation of the 3-butenyl derivative (×), and an extract from winter rape (O). Solvent: See text. The optical densities of both the solutions have been set to 1.0 at 244 mμ. B: The UV-spectra of oxazolidinethiones from a pure preparation of the L-5-vinyl-derivative (×), and an extract from winter rape (O). Solvent: Diethyleter. The optical densities of both the solutions have been set to 1.0 at 248 mμ.

mixed with 10 ml of 0.9 N NH_3 in absolute ethanol in an Erlenmeyer flask, and then kept at room temperature for 24 h. The optical density is measured at 244 mμ in 1 cm cuvettes in a spectrophotometer, against a blank prepared from 25 ml water-saturated 2,2,4-trimethylpentane and 10 ml of the NH_3-solution.

The content of I is calculated as 3-butenyl-isothiocyanate and is determined by comparing the absorption at 244 mμ with the absorption of a sample of pure 3-butenylthiourea at the same wavelength. The molecular extinction coefficient at 244 mμ has tentatively been estimated as 14.0×10^3.

From each of the samples extracted with 2,2,4-trimethylpentane 1 ml is taken and estimated for content of vinyl-oxazolidine-thione according to Wetter.[4]

In order to check the purity of the two extracts obtained from rapeseed meal, their UV-absorption spectra were determined between 220 and 270 mμ and compared with pure preparations of 3-butenylthiourea and L-5-vinyl-2-oxazolidinethione, respectively. The results, as shown in Fig. 1, clearly indicate the close spectral coincidence of the biological extracts and the pure compounds.

Work is in progress in order to study the recoveries with the new method, as well as the optimum conditions for the enzyme reaction.

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