Identification of Sulfur Compounds in Rapeseed Oil[1,2]

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ABSTRACT AND SUMMARY

Seven sulfur compounds were found in industrially extracted rapeseed oils. Four of these were identified as 3-butenyl-, 4-pentenyl-, and phenethyl isothiocyanate, and 5-vinyl-2-oxazolidinethione. These compounds are hydrolysis products from glucosinolates present in the seed.

INTRODUCTION

Sulfur or sulfur compounds present in rapeseed oil have been implicated as catalyst poisons causing hydrogenation difficulties with the oil. Our previous work (1) showed that crude rapeseed oils contained 10-50 ppm sulfur and that this sulfur was mostly removed by the conventional refining procedures. The chemical structure of the sulfur compounds in the oil has not been well established, but it has generally been assumed that they were hydrolysis products from glucosinolates contained in the seed.

Zeman and Zemanova (2) reported 100-200 ppm isothiocyanates (ITCs) in expelled oils and 1800-4600 ppm in extracted oils. Franzke et al. (3) reported 10 ppm ITCs in expelled oil and 100 ppm in extracted oil. They also reported 20 ppm oxazolidinethione (OZT) in extracted oil. Grzybowski, in an earlier study (4), was unable to detect OZT in rapeseed oils. Lanzani et al. (5) reported the presence of allyl- (10 ppm), vinyl- (12 ppm), 3-butenyl- (2.4 ppm), and 4-pentenyl- (1.2 ppm) ITCs in crude rapeseed oil along with 5-vinyl-2-oxazolidinethione (1 ppm), 5-vinyl-thiazolid-2-one (2 ppm), and 2-hydroxy-3, 4-epithiocyanobutane (3 ppm). These compounds were identified by gas chromatographic retention times, by comparison with known compounds. They found none of these compounds in refined rapeseed oils.

In the present study, at least seven different sulfur compounds were found in industrially extracted rapeseed oils. Four of these were identified.

MATERIALS AND METHODS

Materials

Industrially extracted oils were obtained from rapeseed varieties with normal (high) glucosinolate contents (*Brassica campestris*, var. 'Echo,' estimated 21 mg glucosinolates/g oil-free meal, and 'Span,' 19 mg/g). Pure 5-vinyl-2-oxazolidinethione was obtained by courtesy of Dr. L.R. Wetter, National Research Council, Saskatoon, Canada. For reference compounds, allyl-, butyl-, heptyl-, and phenethyl ITC were purchased from Eastman Organic Chemicals (technical grade, Rochester, NY).

Gas Chromatography (GLC)

A Varian Model 1400 gas chromtograph was fitted with a Tracor flame photometric detector (FPD) equipped with a 394-nm filter for operation in the sulfur mode. The FPD has a sensitivity of the order of 10,000:1 for sulfur relative to carbon. It was assumed, therefore, that any major peaks observed were due to sulfur compounds. Since the FPD was mounted on the lid of the chromatograph, it was necessary to install an auxiliary heater in the lid and to use flexible column tubing (Teflon). Columns (1.8 m x 3.2 mm OD) were packed with FFAP on 60/80 mesh Chromosorb W AW DMCS (1:10 w/w), Apiezon L on 60/80 mesh Chromsorb W AW DMCS (3:97), and EGSS-X on 100/120 mesh Gas-Chrom Q (1:99). The FFAP column has been used to analyze isothiocyanates from rapeseed meal (6) while the Apiezon and EGSS-X columns have been used to analyze autolysis products of progoitrin (7). The FFAP and Apiezon columns were programmed at 8 C/min from 100 to 175 C, and the EGSS-X column at 10 C/min from 120 to

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TABLE I

Spray Reagents for Sulfur Compounds on Thin Layer Chromatographic (TLC) Plates

Reagent	Preparation and use	Specificity	Reference
Iodine	Plate explosed to iodine vapors in chamber. Brown spots	Organic compounds	(9)
Silver nitrate	NH_4OH added to 0.1 M $AgNO_3$ to dissolve first precipitate. Spray. Black spots. Plate eventually blackens	Many sulfur compounds	(10)
Grote's reagent	Sodium nitroferricyanide treated with $NH_2OH.HCl$ + $NaHCO_3$, then with Br_2. Blue spots. Unstable	Divalent sulfur doubly linked to a single non-metallic element, e.g., C=S group	(11)
Iodine-azide-starch	Spray with 1% starch, then 1% iodine, then 1% NaN_3. Wite spots on blue background	Thiols, sulfide ion, thioureas, oxazolidinethiones	(12)
$FeCl_3$ + $K_3Fe(CN)_6$	5% $FeCl_3$ + 5% $K_3Fe(CN)_6$ (1:1). Blue spots on yellow background	Substances reducing Fe^{+3}	(8)

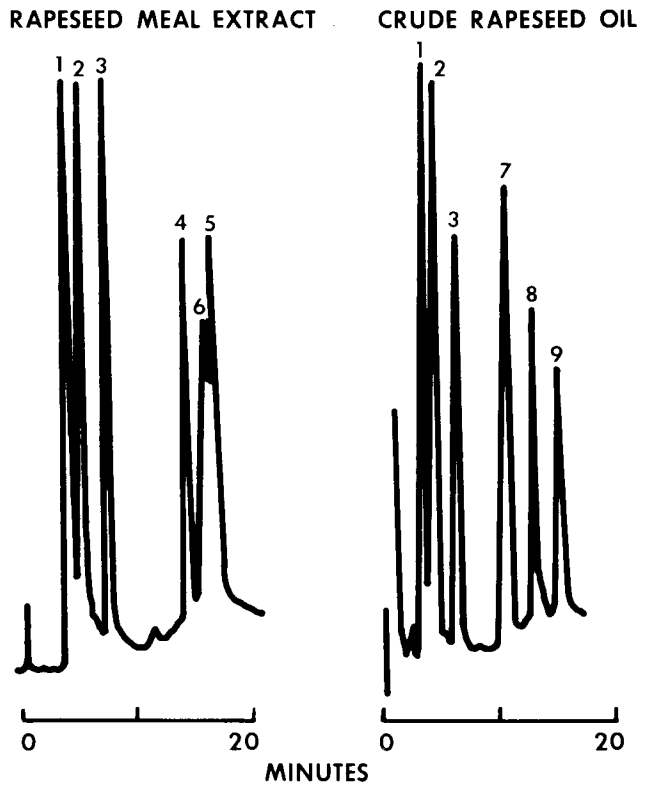

FIG. 1. Gas liquid chromatography of sulfur compounds from rapeseed oil and meal on FFAP column. (1) 3-butenyl isothiocyanate (ITC); (2) 4-pentenyl ITC: (3) heptyl ITC (internal standard); (4) 4-methylthiobutyl ITC; (5) 5-methylthiopentyl ITC; (6) phenethyl ITC; (7,8,9) unknown. Peaks 1, 2, 4, 5, and 6 identified by comparison of retention times with Ref. (6).

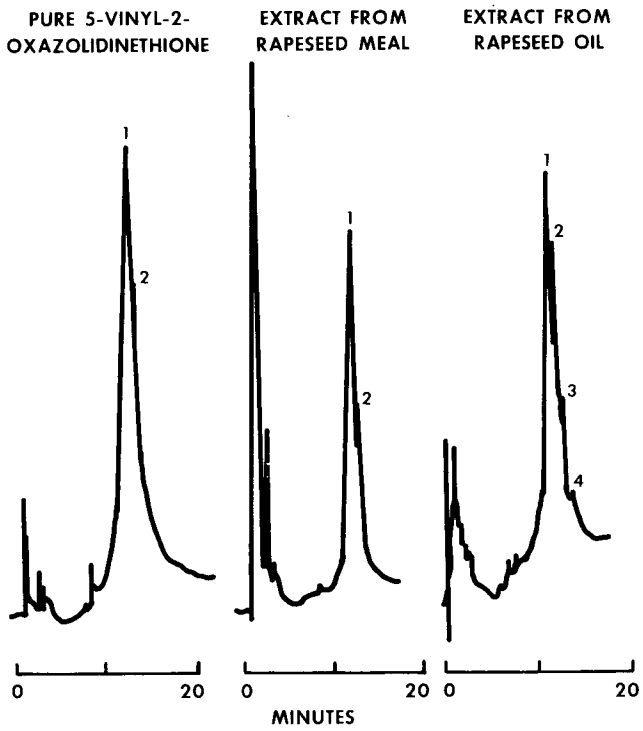

FIG. 2. Gas liquid chromatography of oxazolidinethione (OZT) on EGSS-X column. Peaks 1 and 2 due to 5-vinyl-2-oxazolidinethione.

210 C. The injection port temperature was 220 C, detector 200 C, nitrogen flow rate 40 ml/min, hydrogen and air 50 ml/min, and oxygen 10 ml/min.

A precolumn, used with the FFAP and Apiezon columns for injections of the complete rapeseed oil, retained triglycerides and other oil components that otherwise would foul up the analytical column. The precolumn (15 cm x 3 mm OD glass tubing, slightly flared at the injection end) was packed with 60/80 mesh Chromosorb W AW DMCS, using silanized glass wool plugs. It was attached within the injection port with a 1/8-in. s.s. Swagelok nut, two 1/8-in. silicone rubber o-rings, and a reversed Swagelok back ferrule. The analytical column was attached to the precolumn by a similar connection. The precolumns (at 220 C) lasted for ca. 8 injections of 2 μl oil, when the peaks began to broaden. The precolumns were readily replaced without extinguishing the flame.

Precolumns were not used with the EGSS-X column as the silicone o-rings were destroyed at the higher temperature required for this column. To avoid injection of the total oil, methanol extracts from the oil (5 g oil shaken with 5 ml methanol for 30 sec) were injected (on-column).

Thiourea Derivatives (TUs) of Isothiocyanates

Reference compounds: The purchased allyl-, butyl-, heptyl-, and phenethyl ITCs were individually heated for 16 hr at 45 C with conc. NH_4OH in abs. ethanol (1:1 v/v). After addition of water, the TUs crystallized out; they were recrystallized from water. Their identifies were confirmed by melting point and infrared and mass spectroscopy.

"Meal TU Fraction": An extract of ITCs and OZT from rapeseed (meal) was prepared according to Youngs and Wetter (6) by defatting the heated seed (var. 'Echo'), extracting the myrosinase-treated meal with methylene chloride, and evaporating the solvent. The residual oil extract was heated with NH_4OH-ethanol (1:10) as above. The resulting solution, referred to as the Meal TU Fraction, was used as a source of TU derivatives and OZT from rapeseed meal.

"Oil TU Fraction": ITCs present in the oil were derivatized by heating 500 g oil (var. 'Span,' alkali refined) with 500 ml of NH_4OH-methanol (1:5), for 4 hr at 45 C with stirring. After 48 hr in a separatory funnel, the oil phase was discarded; the methanol phase was washed with 4 x 500 ml "Skellysolve F" petroleum ether and evaporated to dryness. The residual 1 ml of oily material, referred to as the Oil TU Fraction, was used as a source of TU derivatives and OZT from rapeseed oil.

Thin Layer Chromatography (TLC)

Bakerflex media (Silica gel, IB_2 or IB_2F) were used, with the mobile phases (I) chloroform-methanol (10:1), not earlier reported, and (II) the upper phase of ethyl acetate-chloroform-water (30:30:40) according to Ashworth (8). Phase I primarily separated oily material from the ITCs and OZT, and also OZT from the ITCs. Phase II separated the individual ITCs. Spots were visualized by the reagents in Table I. For analytical work, both one-dimensional development (phases I and II separately) and two-dimensional development (phase I followed by phase II) were used. The well-documented identity of the ITCs and OZT in rapeseed meal was confirmed in this way for the Meal TU Fraction by comparison with the TU reference compounds (figures not shown). For preparative work, 10 mg of a sample was applied to a plate in a streak and developed with phase I. The OZT and total ITCs were eluted with methanol. The ITC fraction was redeveloped on another plate with phase II. The bands were eluted with methanol and evaporated to 10 μl for mass spectroscopic analysis.

RESULTS AND DISCUSSION

Samples of rapeseed oil injected via the precolumn into

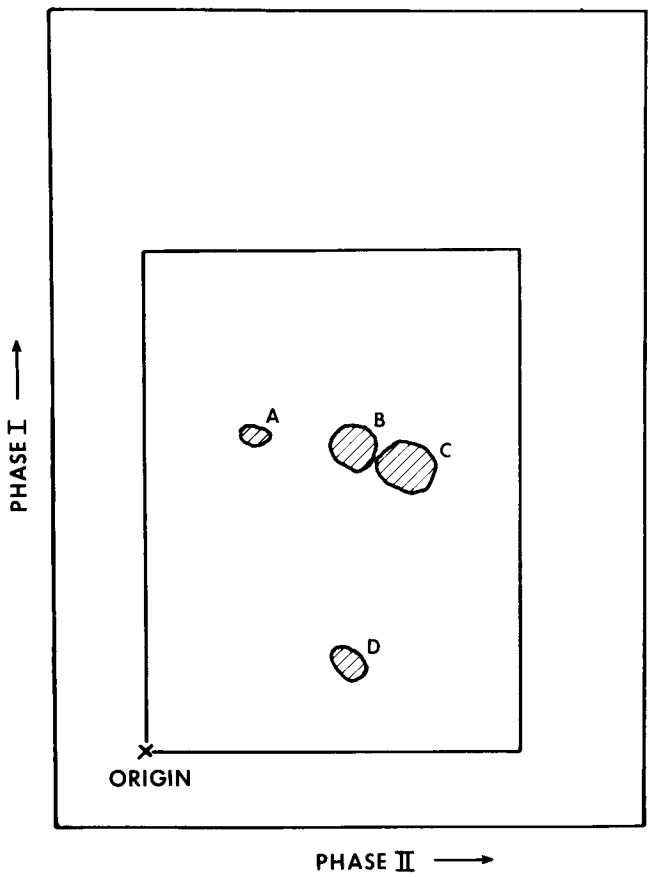

FIG. 3. Thin layer chromatography of rapeseed oil thiourea derivatives (TU) fraction. (A) phenethyl TU; (B) 4-pentenyl TU; (C) 3-butenyl TU; (D) 5-vinyl-2-oxazolidinethione.

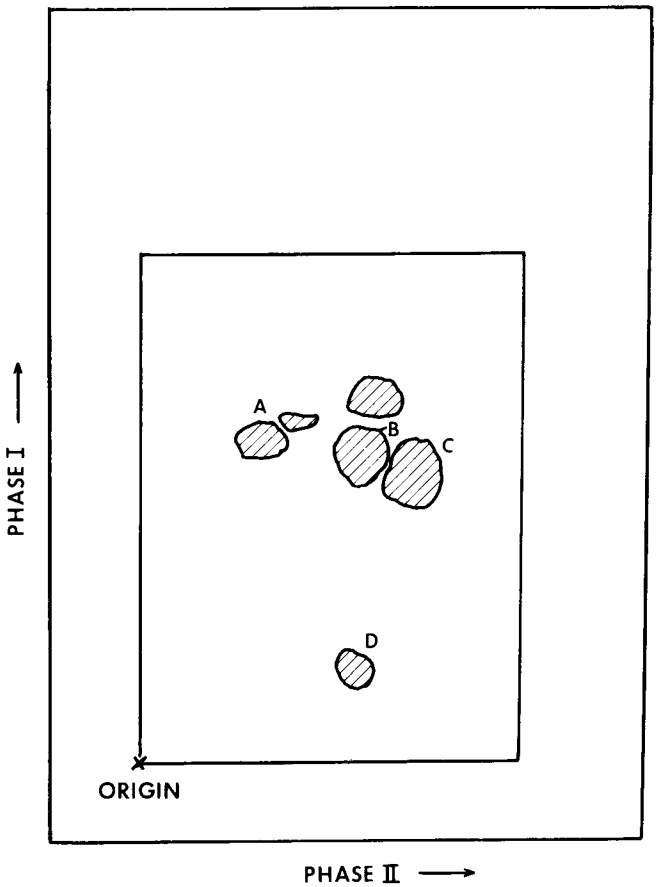

FIG. 4. Thin layer chromatography of rapeseed meal thiourea derivative (TU) fraction. (A) phenethyl TU; (B) 4-pentenyl TU; (C) 3-butenyl TU; (D) 5-vinyl-2-oxazolidinethione.

the FFAP column (Fig. 1) and Apiezon column (not shown) gave five major peaks by the FPD. Several minor peaks, possibly also from sulfur-containing compounds, were not further investigated. Two of the major peaks corresponded in retention time to 3-butenyl- and 4-pentenyl ITC from the chromatographed extract of ITCs and OZT from rapeseed meal (Fig. 1). The other three peaks (No. 7-9) did not correspond to any of the common sulfur-containing hydrolysis products from rapeseed including the epithioalkanes derived from progoitrin reported by Daxenbichler et al. (13). Recently, however, Kirk and MacDonald (14) and Cole (15) have reported the presence of other epithioalkanes in the hydrolysates of unheated rapeseed meal. Further work may show whether the unknown compounds are epithioalkanes.

Methanol extracts of rapeseed oil injected directly into the EGSS-X column gave four peaks by the FPD (Fig. 2). Peaks 1 and 2 corresponded in retention time to the peaks observed from saples of pure OZT and the extract of ITCs and OZT from rapeseed meal (Fig. 2). The presence of two peaks could possibly be explained by partial conversion of 5-vinyl-2-oxazolidinethione to 5-vinyl-thiazolid-2-one in the hot injection port (5). Peaks 3 and 4 may be due to 5-allyl-2-oxazolidinethione whose parent glucosinolate occurs in rapeseed (16) and its thiazolidone counterpart.

To confirm the identity of the compounds revealed by GLC, the Oil- and Meal TU Fractions were analyzed by two-dimensional TLC (Figs. 3 and 4). Four spots from the oil fractions, observed with sulfur-sensitive spray reagents, corresponded in R_f values to the TU derivatives of butenyl-, pentenyl-, and phenethyl ITC and to 5-vinyl-2-oxazolidinethione from the meal fraction. Phenethyl ITC was not observed in the GLC analyses of the oil and might have been masked by one of the unknown compounds in these analyses (cf. Fig. 1).

For mass spectroscopic identification, larger samples of the Oil- and Meal TU Fractions were fractionated by TLC on a preparative scale. Molecular ions were obtained at 130, 144, and 180 AMU for the tentatively identified 3-butenyl-, 4-pentenyl-, and phenethyl TU from the oil, which corresponded to the molecular weights for these compounds. The mass spectra of these compounds were similar to those of the corresponding TU derivatives from the meal. Any differences between the two series of spectra were probably due to impurities in the samples. No mass spectra were obtained for the OZTs from the oil, possibly because these compounds might have decomposed during the concentration procedure.

In conclusion, seven sulfur-containing compounds were found in rapeseed oil. Four of these were identified as 5-vinyl-2-oxazolidinethione and butenyl-, pentenyl-, and phenethyl ITC, mostly by a combination of GLC, TLC, and mass spectroscopy, all by comparison with standards and known compounds isolated from rapeseed meal. The identified compounds are hydrolysis products from glucosinolates present in the seed. The results confirm Lanzani's finding (5) in rapeseed oil of OZT and butenyl- and pentenyl ITC, but not of allyl- and vinyl ITC and hydroxyepithiocyanobutane. Phenethyl ITC has not earlier been reported in rapeseed oil. All seven compounds were found in crude oils and, in smaller amounts, in fully refined and deodorized oils. The quantitative determination of these compounds in various crude and refined rapeseed oils will be reported.

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