Studies On The Degradation Of Glucotropaeolin And Progoitrin - Toxicity And Reactivity Of Splitting Products

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Introduction

To use of rapeseed protein products for human nutrition is not possible today. Purposed technologies of chemical, physical or biotechnological degradation of the high toxic glucosinolates associated with the proteins are not effective enough to produce unharmful food for humans.
Every effective technology has to guarantee the prohibitation of acceptabel daily intake levels of the toxic components. But health limit values are not available neither for the main rapeseed glucosinolate progoitrin (P) nor for its splitting products (e. g. 2-Vinyl-oxazolidin-2-thion, VOT). It is the aim of our work to present principles for the health evaluation of P and some of its often observed degradation products (Fenwick et al., 1983).

Materials and methods

Pure cristalline P (free of VOT) and VOT (m. p. 48 - 50 °C) were isolated from rapeseed (Schnaak, unpublished). Benzyl glucosinolate (BG) was prepared full synthetically (Mr. Junghans, Research Institute Manfred von Ardenne, Dresden, GDR). Special isothiocyanates, ITC (e. g. butenyl-ITC) were prepared by

Dr. Thiel, Technical University Dresden, GDR).
ε-N-alkyl and ε-N-alkenyl amino acids were synthesized in the CIN. Purity, identity and quantity of P and VOT were proofed by HPLC (ASGDR: detection UV_{254}; VOT: Lichrosorb Si60, n-heptane/isopropanol/ethanol = 8/1/1, column 4,6 x 250 mm: P: RP 18, methanol/H_2O = 8/2 tetraalkylammonium salt as ion pair reagent). P was determined after enzymatic splitting with a crude myrosinase preparation (Diedrich, in preparation) and detection of VOT by HPLC (Schnaak et al., in preparation) or GC/MS (Lange et al., 1987). Benzyl-ITC (BZITC) and benzyl nitril (BN) were determined in biological material after methylene chloride extraction by GC (Varian 2100-4: 10 % EGSS-X on Gaschrom P, 100 °C, FID).

Metabolism of benzyl glucosinolate

Male rats (Shoe:Wist) received oral doses of 250 mg BG/kg b. w. by gavage four times in 62 hours in metabolic cages. BG was found mainly in urine and also in the faeces. BN could be identified in kidneys and liver. No BG and splitting products were found in the thyroid gland. But BN and BZITC were also detected in faeces. Only 1 % of BG could be absorbed and unsplitting excreted.

Metabolism of progoitrin

Gnotobiotic male Wistar rats (germfree strain WAG/Rij Rehbruecke: 5 rats per group) were kept in plexiglass isolators and fed commercial basic diet (sterilized by γ-irradiation 25 kGy). 25 to 125 mg P/kg b. w. were given for 3 days. P was excreted via urine and faeces in a greater extend in germfree animals than in conventionalized rats. VOT was not detectable. During incubation of P and VOT separately with the faeces, content of caecum or colon of germfree animals in vitro

no degradation was observable. But incubation of P with microflora of conventional rats results in decreasing of P to 29 - 59 %. VOT concentration did not change. The change of pH resulting in the growth of microorganism is not the reason of P degradation. In no case VOT was detectable as degradation product. So it could be proofed, that intestinal microflora plays an important role in P degradation. If VOT free P is use in the experiments, VOT was not detectable. It seems that the cyclization of hydroxybutenyl ITC to VOT and reactions with active groups of proteins etc. are competetive reactions. In the presence of SH-, NH_2- or other nucleophilic groups it could be possible that no significant amount of VOT are formed (Figure 1). But in this case it is incorrect of reduce the toxicity of P to toxic effects of VOT. Now we try to identify secondary reaction products (other than simple mercapturic acids) of hydroxybutenyl ITC in the organism.

Toxicity of VOT

A 90 day test with isolated pure VOT was carried out to determine the no effect level (noel). Rats (180 ♀, 180 ♂; Shoe:Wist: 112 g b.w. ♀, 106 g b.w. ♂) consumed an average of 1.3, 3.4, 6.7, 12.2 and 1.6 mg VOT/kg b.w./d (10, 25, 50, 100 mg VOT/l drinking water). 34 parameters were determined after 1, 3, 5 or 6 or 7, 12 or 13 weeks and 23 organs are under histological control. The thyroid gland was examined by electron microscopy.

The following selected results should be presented: The absolute and relative thymus weight decreased after dosage of ≥ 25 mg/l (♂) and 100 mg/l (♀) and the absolute and relative liver weight increased (≥ 25 mg/l). The increase in the weight of the thyroid gland was insignificant after 25 and 50 mg/l, and significant at 100 mg/l level. Between male and female rats we found specific differences. Histological changes were detectable in all groups, also in the thyroid gland (25 mg/l). The noel was determined to be < 1,2 mg/kg b.w.. This level was confirmed by a thyroid gland stimulation test with TSH. Changes in T_4 concentration in serum were detectable already in the 10 mg/l group. Determination of the weight of the thyroid gland offers an effect only in the highest concentration. Now search for the exact noel.

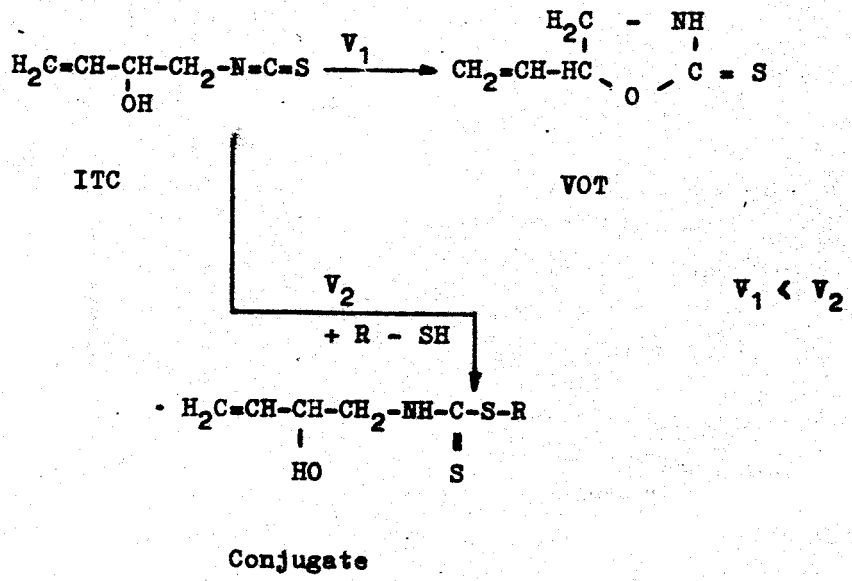

Figure 1: Competetive reactions to VOT and/or conjugates

Reactivity and toxicity of splitting products of glucosinolates

The reaction of ITC with proteins is well known (EDMAN), but the toxicity of ITC-amino acid reaction products is almost unknown (Langer et al., 1964). By differential pulse polarography we get hints that lysine-ε-N-alkyl-thioureas are formed during preparation of rapeseed meal. In modell systems with HSA and rapeseed ITC modell substances the formation of blocked ε-NH_2-groups of lysin and other groups are detectable also by using the indirect dansyl method. No formation was observed in every case below pH 7. This thiourea derivates are unstable in acids. Nobody will found this derivatives after hydrolysis used usually before amino acid analysis. But what happened in practical cases, in which this product are consumed without hydrolysis. What about bioavailability, nutritional value or toxicity of this derivatives. In fact some derivates of alanin (ala) and lysin (lys) are toxic (Table 1) and we make further experiments for risk evaluation after consumtion of this possible secondary protein products of rapeseed crashing and rapeseed protein isolation.

Table 1: Toxic effects of different ITC-amino acid conjugates
(130 /umol N = C = S - group/rat/d)

	thyreotoxic	decreased b. w. gain
Butenyl-ITC	-	-
Butenyl-ala-thiourea	+	+
Butenyl-lys-dithiourea	+	+
Phenyl-ITC (26 /umol)	-	-
Phenyl-ITC (130 /umol)	+	+
Phenyl-ala-thiourea	+	+
Phenyl-ala-thiohydantoin	+	+
Phenyl-lys-ε-thiourea-thiohydantoin	-	-

This investigations are only steps to elaborate health limits of a natural compound and its degradation products. Which is more difficult than in cases of large amounts produced industrial chemicals. But how many native compound we consume daily without good knowledge of health riks and what we have to do after finding out toxic risk in traditional foods?!

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