CO1974COM09

RECENT RESEARCHES ON PROGOITRIN

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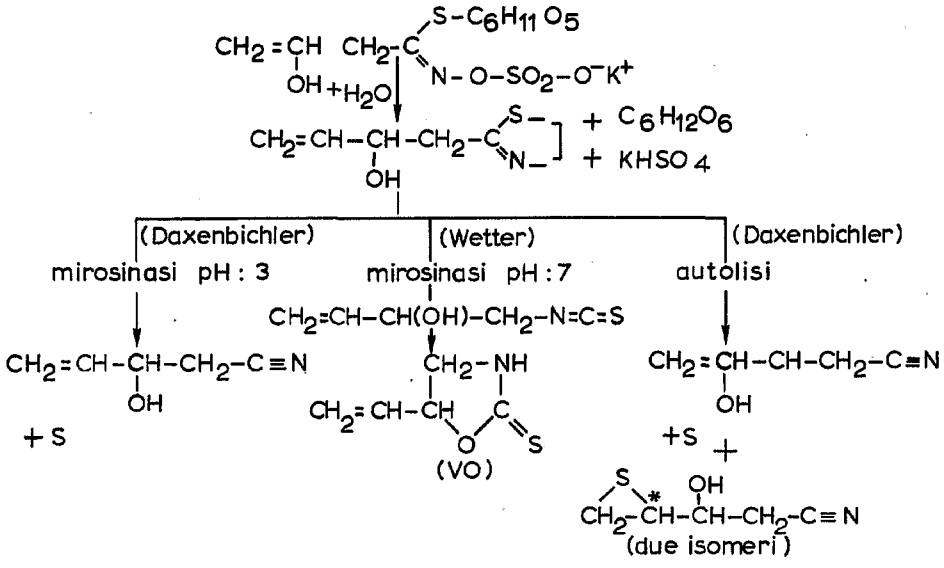
Progoitrine, a thioglucoside contained in the seeds and de-oiled meal of Brassica napus, is known to have toxic effects (through its anti-thyroid, and therefore goitrogenic, action) upon short-stomach animals, while it is virtually harmless for ruminants.

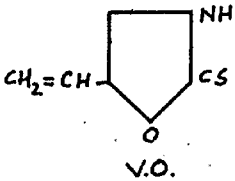
Under the effect of myrosinase- an enzyme found in B. napus - progoitrine is converted into a variety of substances, depending on pH level (GEER, 1962; DAXENBICHLER et al. 1967; LANZANI and JACINI, 1973), the most commonly known of which is 5-vinyl-1.3-oxazolydin-2-Thione (VO).

Figure 1:

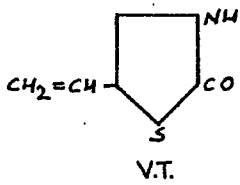


In a previous paper (LANZANI and JACINI, 1971), some of us had reportered that VO, when treated under such conditions as were described by MUKAIYAMA et al. (1966) for 3-phenyl-1.3-oxazolydin-2-thione, undergoes a similar rearrangement, yielding the isomer. 5-vinyl-1.3-thiazolydin-2-one (VT) and a polimer:

Figure 2:



V.O.



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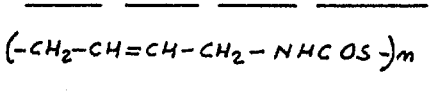

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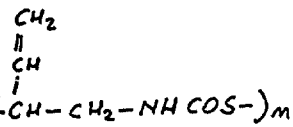


Fig. 2

The purpose of this lecture is to report that VT is also formed from progoitrine (whether pure or contained in B. napus meal) by an enzymatic process when it comes in contact with sheep rumen fluid. In addition to VT and VO, other substances are formed, which are currently being investigated.

We have put sheep rumen fluid in contact at 39.5° C with (i) Brassica meal containing 3.4 % progoitrine, (ii) a mixture of cellulose and progoitrine in the same percentage, and (iii) progoitrine-free alfalfa meal. In a series of experiments, the sheep had been kept on a diet comprising no B. napus, whereas in another series the animal had been accustomed to a diet containing B. napus meal.

After 2 and 24 hours' contact with the rumen fluid, the mixture was lyophilized, then APPELQVIST's method (1967) was used to determine such residual progoitrine as remained in the dry residue as well as the amount of VO formed in the process (residual progoitrine being figured out from the difference between total VO and free VO formed in the process); other transformed products if any were, also investigated. Identical results were obtained as rumen juice was put in contact with pure progoitrine.

Table 1 evidences a gradual decrease in progoitrine, until the substances disappeared altogether after 24 hours' contact with the rumen juice, being replaced by several transformation products: VO, VT and another, still-unidentified substances.

As check test were performed on alfalfa meal under identical conditions, none of the mentioned substances could be isolated.

Table 1: Inoculum from sheep kept on a rapeseed-meal diet (Rapeseed free diet)

% of lyoph. residue	Alfalfa containing substratum			Rapeseed-meal containing substratum		
	0 h	2 h	24 h	0 h	2 h	24 h
Unreacted progoitrine, as VO	0	0	0	0,28 (0,28)	0,02 (0,12)	0 (0)
Newly formed VO	0	0	0	0 (0)	0,02 (0,03)	0,02 (0,09)
VT	0	0	0	0 (0)	0,02 (0,02)	0,08 (0,06)

All data and indication in branckets relate to results obtained from rumen to sheep that had been fed no rapeseed meal.

Table 2:

% of lyophil. residue	Cellulose + 1 % VO		
	0 h	2 h	24 h
VO	0,1 (0,1)	0,01 (0,02)	0 (0,01)
VT	0	0	0

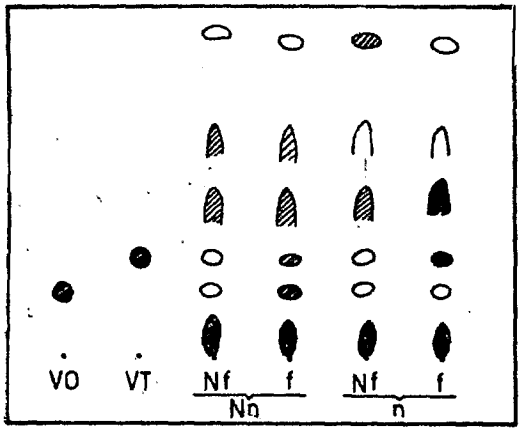
In another sequence of tests, where rumen juice was put in contact with cellulose to which VO had been added, the latter was found to undergo a transformation without however yielding any VT (Table 2). In this case, too, transformation products are currently being investigated.

Such substances as were obtained in the course of all the above reactions were separated by TLC using Hexane-Enthylether (1,1) as the eluent (Fig. 3).

Both VO and VT were identified by IR spectrophotometry using pure samples as reference.
The amounts of VT were determined spectrophotometrically by the typical absorption at 6,0 of this chemical structure (Fig. 4).

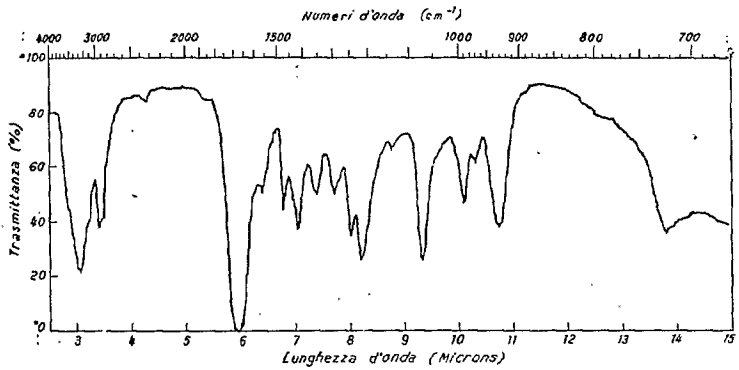
Consequently, the assumption would seem justified that progoitrine is transformed by rumen juice into other substances, amongs which VT prevails. This contention is indirectly substantiated by a recent work of RUTKOWSKI et al. (1973).

Figure 3:



VO; VT	model substances	Nf	2 hours' contact of progoitrine with the rumen fluid
Nn	sheep's diet comprising no B. napus meal	f	24 hours' contact of progoitrine with the rumen fluid
n	sheep's diet comprising B. napus meal		

Figure 4:



References

1. APPELQVIST, L.-A. and E. JOSEFSSON (1967):
I. Sci. Agric. 18, 510

2. DAXENBICHLER, M. E., C. H. van ETTEN, W. H. TALLENT and I. A. WOLF (1967):
Can. J. Chem. 45, 1972

3. GEER, M. A. (1962):
Arch. Biochem. Biophys. 99, 369

4. LANZANI, A. and G. JACINI (1971):
R. I. S. G. 48, 471

5. LANZANI, A. and G. JACINI (1973):
R. I. S. G. 50, 277

6. MUKAIYAMA, T., I. KUWAJIMA and K. MIZUI (1966):
J. Org. Chem. 31, 32

7. RUTKOWSKI, A., H. KOZLOWSKA and J. CWIK (1973):
R. I. S. G. 50, 16