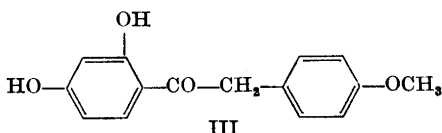


had been: C 67.11; H 4.95; O 27.94. The values found do not agree with the calculated ones. The CO group had thus apparently split off as formic acid on the alkali treatment, why the composition of the compound was $C_{15}H_{14}O_4$, for which the calculated values are: C 69.77; H 5.44; O 24.79. The determination of the methoxy group gave a value corresponding to this. (Found: 11.71 % $-OCH_3$; calc. for $(C_{14}H_{11}O_3) \cdot OCH_3$: 12.02 %.)

The compound reacted with 2,4-dinitrophenylhydrazine. The structure of the compound is, on the basis of the results obtained, the following (III)



This can be formed only if compound A 2 has an isoflavone structure (*i.e.* structure II). The substance isolated by us is thus 7-hydroxy-4'-methoxyisoflavone.

By methylation a methoxy compound is obtained from the ketone III. The m.p. of this compound is 102–104°C. The methoxy determination gave values which correspond to the formula $(C_{14}H_{10}O_3) \cdot (-OCH_3)_2$ (Found: 22.6 % $-OCH_3$; calc. 22.8 %).

In an investigation by Power and Salway³ a compound $C_{16}H_{12}O_4$, m.p. 253°C, was isolated from flowers of red clover. This substance, called pratol, is clearly identical with the substance isolated by us. Structure I has been proposed for it, *i.e.* a flavone structure. On the basis of our investigations this concept has to be corrected. By the method of Robinson and Venkatamaran⁴ we synthesized 7-hydroxy-4'-methoxyflavone and found that it differs from the A 2 substance isolated from clover, both with regard to the melting point and to the UV-spectrum.

The isolated antifungal substances A 1 and A 2 are active against *Sclerotinia trifoliorum* and *Fusarium nivale*. A 1 has a higher activity against *Fusarium* than *Sclerotinia*, A 2 again is much more active against the latter.

This is a preliminary report of our results and further details will be published in a more comprehensive article.

Added in proof: F. Wessely (*Monatshefte Chem.* 57 (1931) 395) has synthesized an

isoflavone, formononetin, which has the same structure as our substance isolated from red clover. Professor Wessely (Vienna) was kind enough to send us a sample of his substance and we could identify it with our substance.

In literature we have found a paper by Cheng *et al.* (*Science* 120 (1954) 575) where determinations of the estrogenic effect of some synthetic isoflavones are presented. Formononetin has also been found to have such an effect.

Mr. P. O. Karvonen has actively taken part in this work, among other things in the determination of the methoxy groups.

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The Transfer of L-5-Vinyl-2-thiooxazolidone (oxazolidinethione) to Milk

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From the seeds of *Brassica* plants Astwood *et al.*¹ have isolated a strongly antithyroid substance which they established as L-5-vinyl-2-thiooxazolidone. In the edible portions of *Brassica* vegetables, such as cabbage, kale, cauliflower, broccoli, mustardgreens, and horseradish root, they did not find this substance. In this laboratory Kreula and Kiesvaara² developed a chromatographic-spectrophotometric method by which vinyl-thiooxazolidone can be determined in green plants in a concentration of 1 μ g/g fresh plants and 5 μ g/litre milk. Contrary to the findings by Astwood *et al.* the present authors found

considerable amounts of vinyl-thiooxazolidone in cabbage, kale, rape, *etc.*, in cabbage for instance 5-40 $\mu\text{g/g}$ fresh weight.

In the literature there has lately occurred information about the antithyroid effect of milk when cruciferous plants are used as fodder³. In order to find out to what extent vinyl-thiooxazolidone is possibly transferred to milk, a feeding experiment was performed with a cow, the daily production of which was 8.2 kg of milk. At 7 o'clock p.m. on the 3rd of March the cow was fed with 0.5 kg of finely ground moistened rape seeds which according to analysis contained 4.5 g of vinyl-thiooxazolidone. The active substance is formed from a glycoside found in the seeds by enzymatic action. In the moistened seeds the liberation takes place rapidly, and this presumably happens in the rumen too. A milk sample was taken before feeding, 12 h after feeding, and then every 12 h for 6 days.

Not even a trace of vinyl-thiooxazolidone, which has a strong absorption maximum at 240 $m\mu$, was found in any milk sample. On the other hand, a small amount of a substance with a strong absorption maximum at 255 $m\mu$ was found in the milk 12 h after feeding (after 24 h the amount was already very small). After 48 h there were only traces of it in the milk (Fig. 1).

Since strong reductive processes take place in the rumen — among other things unsaturated fatty acids are largely reduced to saturated ones — it is possible that vinyl-thiooxazolidone is reduced to the corresponding 5-ethyl-2-thiooxazolidone in the rumen. The substance excreted into milk could thus be this compound. According to Astwood *et al.* the spectra of 5,5-dimethyl-2-thiooxazolidone and 2-thiooxazolidone are nearly identical with the spectrum of 5-vinyl-2-thiooxazolidone. On the basis of this, the side chain would have no greater influence on the spectrum of compounds of this type, and the substance found in milk would accordingly not be ethyl-thiooxazolidone. The amount of the substance excreted into milk was minimal, about 0.1 % of the vinyl-thiooxazolidone given to the cow, provided that the specific absorption of the substances at 240 and

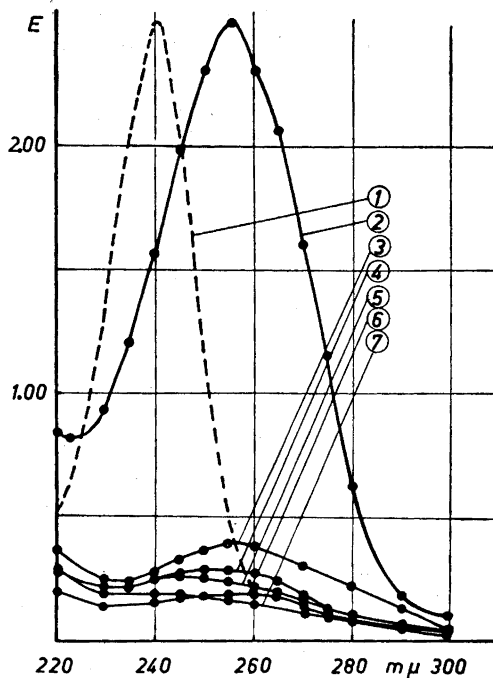


Fig. 1. UV-spectra of the compound which appears in cow's milk after feeding of 0.5 kg of finely ground moistened rape seeds containing 4.5 g of L-5-vinyl-2-thiooxazolidone (2—7). The curves show the relative values after 12 h (2), 30 h (3), 54 h (4), 78 h (5), 102 h (6), and 126 h (7). The dashed curve (1) shows the spectrum of L-5-vinyl-2-thiooxazolidone.

255 $m\mu$, respectively, is approximately the same. The investigations concerning the substance found in milk continue.

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