

The degrees of dissociation at room temperature calculated by means of the data in columns Nos. 7 and 8 are in column 9. The mean value is 0.18. Klemm and Pauli⁵ report 0.23 at 20°C. Their susceptibility values at two lower temperatures may be interpreted as indicating a decrease in the degree of dissociation with decreasing temperature.

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Isolation of an Anti-Sclerotinia Factor, 7-Hydroxy-4'-methoxyisoflavone from Red Clover

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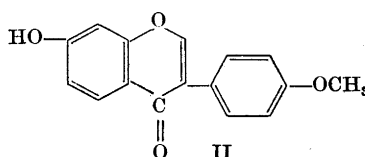
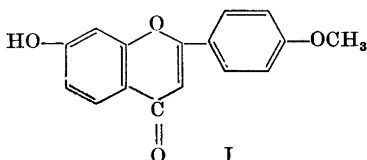
When investigating the antifungal substances in red clover, we isolated a new compound (A 1) the characteristics of which we have earlier reported^{1,2}. The structure elucidation of this compound is still in progress. When isolating this compound, we also came to isolate an other substance (A 2) which is found in comparatively large amounts in the leaves and the stems

of red clover. From fresh clover plants the isolation and separation of the two substances in pure form was difficult, since on paper chromatograms both substances behaved in much the same way in different solvent systems. The separation succeeded, however, using O'Keeffe fractionation. 2.69 g (m.p. 217°C) of A 1 was obtained in crystalline form from 10.75 kg of fresh red clover and 4.6 g (m.p. 262°C) of A 2, likewise in crystalline form. A large amount of the latter remained in the motherliquor, however. The total amount of A 2 was calculated on the basis of paper chromatography from the fluorescence of the spots to about 10 g per 10 kg fresh clover harvested 26.7. Since A 1 disappears on drying of the clover, the isolation of A 2 from dried clover is easy (ether extraction, extraction of the ether residue by petroleum ether and sublimation of the undissolved residue *in vacuo* at 260 to 280°C).

The structure of A 2. Elementary analysis: C 71.89; H 4.66; Calc. for C₁₆H₁₂O₄: C 71.63; H 4.51. The substance contained one hydroxy and one methoxy group. The content of the methoxy group in the substance was 11.1 %, determined by the method of Vieböck and Brecher, calculated for the compound (C₁₅H₉O₃)-OCH₃ 11.57 %.

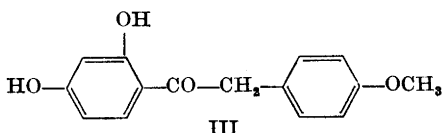
Using Rossman's method the substance in 30 min took up an amount of bromine corresponding to one double bond. In the alkali melt (131 mg of A 2, 2.74 g of solid KOH, 230–250°C, 2 h) 2,4-dihydroxybenzoic acid and *p*-hydroxybenzoic acid were formed. When heated in BaOH₂ solution anisic acid was formed. The structure of the A 2 compound accordingly is I or II.

On heating 400 mg of the substance in NaOH solution on a water bath for 20 min a substance was obtained after neutralisation with hydrochloric acid, acidification with acidic sodium phosphate and extraction by ether. This substance crystallized in flaky crystals and melted at 164°C. The yield was 285 mg. The elementary analysis of the substance was C 69.36; H 5.61; O 24.62. If the compound formed had been a chalcone (C₁₆H₁₂O₄ + H₂O) corresponding to A 2, the calculated values



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