Effect of some Metal Ions on the Stability of Natural Vitamin A

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Metal ions have been shown to accelerate the oxidation of fat even when present in very small concentrations. As vitamin A is destroyed when the fat in which it is contained turns rancid through oxidation, some experiments have been carried out to investigate the concentrations of some metal ions required to cause a measurable acceleration of the vitamin A destruction.

Fish liver oil concentrates*, containing about 100 000 IU of vitamin A per g, were used in the experiments. The metal ions of the oil samples were removed by percolating a solution of equal parts of oil and propanol-2 (with some benzene added to ensure complete miscibility) through a cation exchanger in the hydrogen form. Solutions of metal chlorides in absolute ethyl alcohol were then added to the samples so that a final concentration of 2 % ethyl alcohol and 1, 10, or 100 parts per million (ppm) of metal ion was obtained. The metals used in these experiments were Al, Cu, Fe, Ni, Sn and Zn. The samples were distributed in a number of

vials which were stored for different lengths of time at a temperature of 34.5° C according to the Schaal oven test modified by Simons *et al.*¹. At intervals two vials of each sample of oil were removed from the oven and individual determinations of the vitamin A content were immediately made.

Vitamin A was determined by measuring the absorption at 312 m μ , 325 m μ (or the absorption maximum in the interval 325 m μ —328 m μ), and 334 m μ of a solution of the oil in hexane, purified by percolation through a column of defatted bone meal according to Dowler and Laughland². The absorption was corrected using the Morton-Stubbs equation and the content of vitamin A was calculated using a conversion factor of 1 900.

Table 1. The figures in the table represent the ratios of the time for 50 % destruction of vitamin A in oils containing various concentrations of metal ions, to the time for the same destruction of vitamin A in oil containing no metal ions.

Metal ion	Concentration of metal ion, ppm		
	1	10	100
Al	0.9	0.7	0.5
Cu	0.8	0.5	0.5
Fe	1.0	0.7	0.2
Ni	0.9	0.7	0.4
Sn	0.9	0.9	0.7
Zn	0.9	0.9	0.8

* The oil samples were kindly supplied by Kjellbergs Successors AB, Stockholm.

Fig. 1. Destruction of vitamin A in oil from which metal ions had been removed and to which various amounts of Al had been added (as AlCl_3). The samples were kept in the dark and exposed to air at 34.5° C for different lengths of time.

○ ○ Oil, (metal ions removed)
 × ——— × " " " " + 1 ppm Al
 △ - - - - △ " " " " + 10 ppm Al
 ● - · - · ● " " " " + 100 ppm Al

A typical diagram showing the percentage destruction of vitamin A relative to time is shown in Fig. 1.

The results of the experiments are summarized in Table 1.

It should be emphasized that the figures given in Table 1 are only approximate as they depend on several factors, e. g. variations in the composition of different batches of oil, and the storage conditions before testing.

1. Simons, E. J., Buxton, L. O. and Colman, H. B. *Ind. Eng. Chem.* **32** (1940) 706.
2. Dowler, M. W. and Laughland, D. H. *Anal. Chem.* **24** (1952) 1047.

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*iso*Thiocyanates XVIII. Glucocapparin, a New Crystalline *iso*Thiocyanate Glucoside

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A previous paper of this series ¹ described the detection of methyl *isothiocyanate* as a new natural mustard oil, present in various species of the family *Capparidaceae* as a glucoside, provisionally named *glucocapparin*. We now wish to report on the isolation and characterization of glucocapparin in crystalline condition.

The new glucoside was readily obtained as colourless needles from an extract of seeds of *Cleome spinosa* Jacq., purified as described in the experimental part. The analytical data indicated the composition $C_8H_{14}O_6NS_2K$ for glucocapparin which is the formula expected for a glucoside containing methyl *isothiocyanate* in the traditional combination with sulphuric acid, potassium and glucose. When glucocapparin was subjected to enzymic hydrolysis, methyl *isothiocyanate* was liberated and could be identified as *N*-methylthiourea after distillation and treatment with ammonia. The concomitant formation of sulphate and glucose was demonstrated, the latter by paper chromatography in two solvent systems. The ultra-violet absorption spectrum of glucocapparin displayed a maximum at 225 $m\mu$ (ϵ 9 850) in aqueous solution, very similar to the spectra determined in this laboratory for other mustard

oil glucosides (Refs. ^{2,3} and unpublished data). Again, the infra-red absorption spectrum was closely comparable to the spectra of sinigrin ⁴ and glucoiberin ⁵, re-determined in this laboratory, a fact suggesting that glucocapparin has a chemical structure closely related to that of other mustard oil glucosides.

For the purpose of further characterization glucocapparin was transformed into a crystalline tetraacetate. That this process was not accompanied by structural changes appeared from the fact that deacetylation with ammonia afforded unchanged glucocapparin as established by the infra-red spectrum and paper chromatography. Recently, acetylation has been successfully employed by Schultz and Wagner ⁶ for characterizing several mustard oil glucosides as crystalline derivatives.

Glucocapparin adds another representative to the still rather limited group of crystalline *isothiocyanate* glucosides, heretofore comprising sinigrin, sinalbin, glucocheirolin ⁷ and glucoiberin ⁵. The new glucoside is remarkable in being the first of this type crystallizing without water of crystallization. So far, its known occurrence is limited to species of the family *Capparidaceae*.

Experimental. Isolation. Finely ground seeds (160 g) of *Cleome spinosa* * were extracted with ligroin, containing some isopropanol, in order to remove fatty material. The residue (107 g) was subjected to exhaustive extraction, first with 70 % methanol (800 ml) and then with pure methanol (300 ml). The extracts were pooled, a precipitate removed by filtration and the solution concentrated to about 150 ml *in vacuo*. After removal of a new precipitate, the solution was rapidly sucked through a column of neutral aluminium oxide (40 g) in order to remove further impurities. The column was washed with water (100 ml) and the joined filtrate and washing liquid was allowed to flow slowly through another column containing 80 g of anionotropic alumina (Woelm) which quantitatively retained the glucoside ⁴. The column was washed with water and then eluted with a 1 % solution of potassium sulphate. The end-point was conveniently estimated by the appearance of a brownish colour in the eluate, emerging simultaneously with the first sulphate ions. The eluate was taken to dryness *in vacuo* and the glucoside separated from traces of inorganic material by repeated

* Commercially obtained from E. Benary, Hann.-Münden, Germany.