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.

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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 *iso*thiocyanate 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 $C_8H_{14}O_9NS_2K$ for glucocapparin which is the formula expected for a glucoside containing methyl *iso*thiocyanate in the traditional combination with sulphuric acid, potassium and glucose. When glucocapparin was subjected to enzymic hydrolysis, methyl *iso*thiocyanate 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 mμ (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 [2,3] and unpublished data).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], redetermined 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 *iso*thiocyanate 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 *iso*propanol, 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.

extractions with hot methanol. The organic solvent was removed, leaving a colourless lac which was dissolved in hot 80 % ethanol. Upon cooling and scratching crystals separated (330 mg); pure glucocapparin (271 mg) was obtained as glistening, colourless needles after recrystallization from 80 % ethanol. The above procedure could most likely be modified to give an improved yield of glucocapparin, but no systematic studies were performed with this aim.

Properties of glucocapparin. Glucocapparin decomposes gradually above 198°. Its rotation was determined in aqueous solution, $[\alpha]_D^{25}$ —28.6° ± 1.5° (c = 2.2). (Found: C 25.90; H 3.96; N 3.71; S 17.47. Calc. for $C_8H_{14}O_9NS_2K$: C 25.86; H 3.80; N 3.77; S 17.27). The infra-red spectrum, determined in a KBr pellet, displayed prominent bands at 2.80 (v.s., OH-stretching), 6.28 (m.), 6.93 (m.), 7.17 (w.), 7.31 (w.), 7.51 (m.), 7.72 (s.), 7.82 (s.), 8.14 (v.s., broad), 8.87 (w.) *, 9.00 (w.) *, 9.16 (s.), 9.34 (v.s.), 9.46 (v.s.), 9.74 (s.) **, 10.87 (m.), 11.27 (s.) and 12.43 μ (v.s.).

Glucocapparin (50 mg) was dissolved in a phosphate buffer of pH 6.6 (2 ml) and a drop of a myrosinase solution was added. Next day, the *iso*thiocyanate was removed by distillation and transformed into its thiourea-derivative with ammonia. The thiourea was identified as *N*-methylthiourea on paperchromatographic comparison with an authentic specimen in two solvent systems, *viz.* water-saturated chloroform and *n*-butanol-ethanol-water (4:1:4). Furthermore, the ultra-violet absorption spectrum coincided with that of authentic *N*-methylthiourea. The latter exhibited a maximum at 235 mμ (ε 11 900) and a minimum at 218 mμ (ε 6 300) in water.

An enzymically hydrolyzed solution of glucocapparin (5 mg) was taken to dryness and the residue extracted with methanol. The insoluble part was dissolved in water and the presence of sulphate ions demonstrated as $BaSO_4$. The methanolic extract was chromatographed on paper in two solvent systems, *viz.* *n*-butanol-ethanol-water (4:1:4) and pyridine-amyl alcohol-water (35:30:30), and the presence of glucose established by comparison with authentic specimens.

Glucocapparin-tetraacetate. A solution of glucocapparin in dry pyridine and excess acetic anhydride was kept at 50° for 3 hours. A large volume of ether was added to the pink solution, resulting in precipitation of the crystalline acetyl-derivative, which was recrystallized twice from methanol and separated as tiny, colourless needles, m. p. 209—210° (uncorr., decomp.); $[\alpha]_D^{25}$: —31.0° ± 1.5° (c = 1.82, water). (Found ***: C 35.63; H 4.21; N 2.42; S 11.68. Calc. for $C_{16}H_{22}O_{13}NS_2K$: C 35.62; H 4.21; N 2.60; S 11.88). In aqueous solution the ultra-violet absorption spectrum of the tetraacetate was similar to that of glucocapparin, possessing λ_{max}: 221 mμ (ε_{max} 9 340)[4]. The infra-red spectrum (in KBr) had conspicuous bands at: 5.72 (v.s., ester CO-band), 6.93 (m.), 7.24 (s.), 7.70 (s.), 8.09 (v.s., broad), 9.12 (m.), 9.37 (v.s.), 9.50 (s., plateau), 10.92 (m.), 11.23 (s.) and 12.90 μ (s., broad).

Glucocapparin-tetraacetate (60 mg) was dissolved in anhydrous methanol, saturated with ammonia at 0°, and kept at room temperature. Next day, the solvent was removed and the residue recrystallized from aqueous ethanol to give glucocapparin (33 mg, 80 %), identified by its infra-red spectrum and paperchromatographic comparison. R_F-values of 0.06 and 0.44 were determined in descending paper chromatograms at 24° on Whatman paper No. 1 for glucocapparin and its tetraacetate, respectively, with *n*-butanol-ethanol-water (4:1:4) as a solvent system.

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* Probably C—O-stretching- (or OH-deformation-)modes of secondary OH-groupings; the bands disappear on acetylation.

** Probably C—O-stretching-(or OH-deformation-) mode of the primary OH-grouping; the band disappears on acetylation.

*** As also experienced elsewhere [6], attempted O-acetyl determinations on this and other glucoside acetates consistently gave too high values.

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