

Fig. 3. Same as Fig. 2A, but tyrosine (spot 7) is added to the chromatogram.

found too. The amino acid is certainly not identical with tyrosine (Fig. 3).

In kidney about the same amounts of keto-acids were found as in muscle (calculated on fresh wt.), but in liver only 1/8 of this quantity. In all cases hydroxypyruvic acid was found, as mentioned earlier. Thus this acid is now for the first time shown to exist free in animal tissues. However Sprinson and Chargaff⁷ noticed that hydroxypyruvic acid is formed in slices of rat kidney as an oxidative deamination product of serine.

In some cases small spots of threonine (from β -hydroxy α -ketobutyric acid) and tyrosine (from phenylpyruvic acid) were found, but the existence of the corresponding keto-acids in nature could, however, not be proved because of the poor material at our disposal.

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isoThiocyanates XIII. Methyl iso-Thiocyanate, a New Naturally Occurring Mustard Oil, Present as Glucoside (Glucocapparin) in *Capparidaceae*

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In a previous communication of this series¹ attention was given to a thiourea of extraordinarily low R_{Fh} -value (0.03)², derivable from the volatile isothiocyanate fraction of certain seed samples. The mustard oil was tentatively regarded as methyl isothiocyanate which had not previously been encountered in Nature. This suspicion has now been confirmed by the isolation of methylthiourea from the ammonia-treated distillate of extracts of seeds of *Cleome spinosa* Jacq. (*C. pungens* Willd.) after enzymatic hydrolysis.

The older literature³ reports the use of various members of *Capparidaceae* as spices and remedies in folk medicine. These applications have been partly attributed to the contents of volatile constituents of unknown structure. In a previous paper¹ the species *Cleome arabica* L. and *Gynandropsis gynandra* (L.) Briq. were listed as sources of the presumed methyl isothiocyanate, the latter being particularly rich in its contents. Methanolic extracts of various species of *Capparidaceae* have now been submitted to paperchromatographic investigation of their content of isothiocyanate glucosides, essentially by the method of Schultz and Gmelin^{4,5}. The results are schematically reproduced in Fig. 1. It should be noted that two glucosides with widely differing R_F -values appear

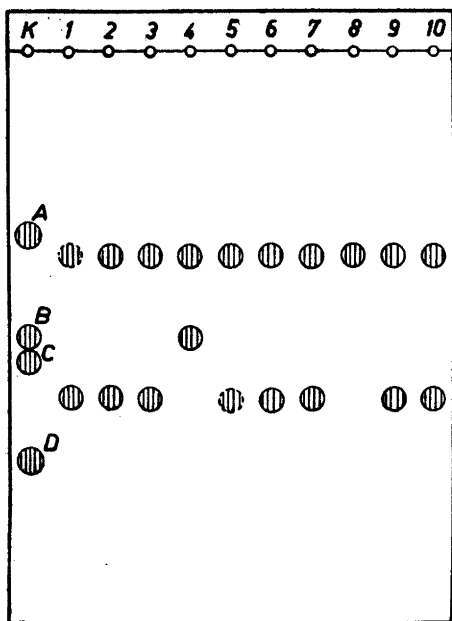


Fig. 1. Schematic, descending paper chromatogram of isothiocyanate glucosides in various Capparidaceae. Solvent system: *n*-butanol:acetic acid:water (4:1:3). *K* represents a control mixture of: A) glucoiberin⁵ B) sinigrin; C) sinalbin; D) glucotropaeolin. The following species are represented:

- | | |
|-----------------------------|-----------------------------|
| 1. <i>Cleome arabica</i> L. | 8. <i>Gynandropsis</i> |
| 2. <i>C. arborea</i> Bes. | <i>gynandra</i> (L.) |
| 3. <i>C. gigantea</i> L. | Brig. |
| 4. <i>C. monophylla</i> L. | 9. <i>Cleome graveolens</i> |
| 5. <i>C. speciosissima</i> | Rafin. |
| Deppe. | 10. <i>C. trachysperma</i> |
| 6. <i>C. spinosa</i> Jacq. | (Torr. & Gray) |
| 7. <i>C. viscosa</i> L. | Pax & K. Hoffm. |

to represent the general pattern of this family, occasionally accompanied by an additional glucoside as in, e. g., *C. monophylla* L. The relative amounts of the two glucosides in various species show some variation but generally they appear to be present in about equal amounts.

When a larger sample of seeds of *C. spinosa* Jacq. (125 g) became available it was treated with myrosinase whereafter a sharp and typical smell of mustard oil soon developed. The water-soluble oil was removed

by steam distillation and collected in ammonia. After standing, the solution was concentrated to dryness *in vacuo* leaving a semi-crystalline residue (290 mg), which was transformed into beautiful, dense crystals upon three recrystallizations from acetone. M. p. 121° (uncorr.), alone or in admixture with an authentic specimen of *N*-methylthiourea. (Found: C 26.54; H 6.43; N 30.84. Calc. for $C_7H_8N_2S$: C 26.65; H 6.71; N 31.09).

For the glucoside, yielding methyl isothiocyanate on enzymatic cleavage, we should like to suggest the name *glucocapparin*. The fact that *C. monophylla* L., lacking the second main glucoside, on cleavage, distillation and treatment with ammonia also afforded methylthiourea proved the lower glucoside-spot of Fig. 1 to be attributable to glucocapparin, a constant constituent throughout the botanical family. The chemical nature of the second characteristic glucoside of higher R_F -value as well as of the second component in *C. monophylla* is at present being further investigated. It is obvious, however, that both substances represent glucosides yielding non-volatile mustard oils on enzymatic hydrolysis.

No definite proof of the presence of glucocapparin in *Cruciferae* has yet been found. The examples previously listed¹ have needed some revision which will be presented in forthcoming communications.

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