isoThiocyanates XXV*. Methyl 4-isoThiocyanatobutyrate, a New Mustard Oil Present as a Glucoside (Glucoerypestrin) in Erysimum Species

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A few years ago it was noticed in this laboratory 1 that seeds of various species of the genus Erysimum (Cruciferae) contain a mustard oil glucoside which is different from those formerly recognized in Nature. A substantial seed sample of Erysimum rupestre DC., procured by cultivation of the plant on a larger scale **, has now made it possible to characterize and identify the new glucoside for which we wish to introduce the name glucoerypestrin ***.

According to paperchromatographic analysis, glucoerypestrin is accompanied by minor amounts of glucocheirolin in seed extracts of E. rupestre. The partly purified, glassy glucoside fraction yielded a crystalline product on acetylation. Fractional crystallization of this from 90 % ethanol afforded a homogeneous specimen of glucoerypestrin tetraacetate monohydrate, decomposing at $188-190^\circ$ (uncorr.) †; $[a]_D^{13} - 18.0^\circ$ (H_2O , c = 1.7). (Found ††: C 37.40; H 4.85; H 2.05; H 6.19. Calc. for $C_{20}H_{28}O_{15}NS_2K$, H_2O (643.7): H 37.32; H 4.70; H 2.18; H 6.07). The infra-red spectrum was of the usual type H 3,4 and did not provide much information as to structural details.

Deacetylation with 1.5 N methanolic ammonia, under carefully controlled con-

ditions, furnished the alkali-labile, paperchromatographically homogeneous glucoerypestrin which, in turn, was subjected to enzymic hydrolysis with myrosinase at pH 6.7. The resulting mustard oil, isolated by ether extraction or distillation at diminished pressure, yielded a crystalline, optically inactive thiourea (Ha), C₄H₁₂O₂N₂S, m.p. 93° (uncorr.) (Found: C 40.85; H 6.83; N 15.65; S 18.60; OCH₃ 17.58. Calc.: C 40.90; H 6.87; N 15.90; S 18.20; OCH₃ 17.61), on treatment with methanolic ammonia, again under strictly controlled conditions. The ultra-violet spectrum of the thiourea (in EtOH) was of the usual type (λ_{max} 243 m μ (ε 12600), λ_{min} 223 m μ (ε 1500). A very strong band at 1728 cm⁻¹ in the infra-red spectrum (in KBr) was suggestive of the presence of an ester grouping in the thiourea. On paper chromatography in water-saturated chloroform, the new thiourea travelled at a rate corresponding to an Rph-value of 0.65 5.

Reaction of the isothiocyanate with aniline and 1-naphthylamine in methanolic solutions afforded the nicely crystalline phenylthiourea (IIb), C₁₂H₁₆O₂N₂S, m. p. 91° (uncorr.) (Found: C 57.00; H 6.39; N 11.01. Calc.: C 57.12; H 6.39; N 11.11), and (1-naphthyl)-thiourea (IIc), C₁₆H₁₈O₂N₂S, m. p. 132° (uncorr.) (Found: C 63.70; H 5.85; N 9.03. Calc.: C 63.56; H 6.00; N 9.27), respectively.

SCNCH₂CH₂CH₂COOCH₃

IIa: R = H $IIb: R = C_6H_5$ $IIe: R = 1 \cdot C_{10}H_7$

The cumulate data suggested that the new mustard oil was methyl 4-isothiocynato-butyrate (I), an assumption fully verified through the following synthesis.

temperature at a rate of 8° per min.

Polymorphism may also in some cases contribute to the widely varying values observed (M. G. Ettlinger, private communication).

†† A Karl Fischer titration gave: 2.85 %

H₂O. Calc. for the monohydrate: 2.80 %.

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^{**} We are much indebted to the Botanical Garden of the University of Copenhagen for the cultivation of this and other biennial Erysimum species.

^{***} This name has been preferred to the more consistent designation "glucoerysimin" in order to avoid confusion with the cardiac glycoside erisimin of similar provenance (cf. Ref.²).

[†] It has been observed in this laboratory that the decomposition temperature of most acetylated *iso*thiocyanate glucosides is very dependent on the rate of heating. The present value was determined in capillary tubes by inserting the sample at 140° and reising the temperature at a rate of 8° per min.

Treatment of 4-aminobutyric acid with hydrogen chloride in anhydrous methanol gave a quantitative yield of methyl 4-aminobutyrate hydrochloride * as hygroscopic needles, m. p. 96—99° (uncorr.) (Found: C 38.70; H 7.69; Cl 22.71. Calc. for C₅H₁₂O₂NCl (153.6): C 39.09; H 7.88; Cl 23.08). Equimolecular amounts of thiocarbonyl chloride in chloroform, and the hydrochloride, dissolved in water containing excess sodium carbonate, reacted to give the mustard oil (I) as an almost colourless oil, b. p. 70° at 0.2 mm, $n_{\rm D}^{28}$ 1.5066. (Found: C 45.40; H 5.82; N 8.77; S 20.22. Calc. for $C_6H_9O_2NS$ (159.2): C 45.26; H 5.70; N 8.80; S 20.14). Upon reaction with ammonia, aniline and 1-naphthylamine, respectively, the synthetic mustard oil yielded thioureas (IIa, b, c) identical with those obtained from the natural isothiocyanate, as apparent from mixed melting point determinations and coinciding infra-red spectra.

The presence in glucoerypestrin of glucoese and sulphate has been unequivocally established by paper chromatography subsequent to enzymic hydrolysis. In accord with the notable discovery of Ettlinger and Lundeen, leading to a revised general structure for the mustard oil glucosides, glucoerypestrin splits off hydroxylamine on hydrolysis with strong acids. Hence, the formula (III) is considered likely to be a correct expression for the glucoerypestrin anion.**

same glucoside pattern as *E. rupestre* DC. In a recent survey of paperchromatographic analyses of a considerable number of plants, Schultz and Wagner ⁸ list the *R_B*-values 0.65 and 0.68 in *n*-butanol: acetic acid: water (4:1:3) and *n*-butanol: pyridine: water (6:4:3), respectively, for a glucoside present in *E. pumilum* DC. and *E. rupestre* DC. *f. aurantiacum*. There can be little doubt that this glucoside is glucoerypestrin.

A detailed account of the present work, together with some biogenetic considerations, will form the subject of a forthcoming publication.

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Of several *Erysimum* species investigated thus far in this laboratory, seeds of *E. ochroleucum* DC. and *E. pumilum* DC. exhibited the

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^{*} No physical constants or analyses of this salt can be found in the literature, although it appears that Spāth *et al.*⁶ have employed the compound for further syntheses.

^{**} The stereochemistry of the oxime linkage has not yet been definitely established.