

by treatment with excess of 0.1 N NaOH and back-titration by 0.1 N HCl. Hydrolysis of the crude polysaccharide mixture (2 N H<sub>2</sub>SO<sub>4</sub>, 18 h, 100°) gave the monosaccharides of Table 1; identified by chromatography in (A), (B), (C), and by measuring  $[\alpha]_D$  after separation of thick paper.

*Degradation with pectinase.* Crude polysaccharide was hydrolysed (1 N H<sub>2</sub>SO<sub>4</sub>, 6 h, 100°), neutralised with BaCO<sub>3</sub>, and separated on De-Acedite (formate form) in a neutral and an acid fraction. The latter was incubated for 18 h at 37° with pectinase (pure, Sigma).

The enzyme was removed by heating a few minutes at 100° and filtered through Millipore Membrane filter. Galacturonic acid and small amounts of galactose and arabinose were liberated. Incubation of crude polysaccharide with pectinase under the same conditions as above gave galacturonic acid, galactose, glucose, and arabinose as main components.

*Fractionation of the crude polysaccharide* was carried out on DEAE cellulose (Whatman DE 11). The polysaccharide (0.1–0.2 g) was dissolved in water and poured in a column (2 × 30 cm) in phosphate form.<sup>9</sup> The column was eluted as indicated in Fig. 1. The composition of the various fractions is given in Table 1.

1. Jones, J. K. N. and Smith, F. *Advan. Carbohydrate Chem.* **4** (1949) 264.
2. Steinegger, E. and Hänsel, R. *Lehrbuch der allgemeinen Pharmakognosie*, Heidelberg 1963.
3. Spinall, G. O. and Molloy, J. A. *J. Chem. Soc.* **1968** 2994.
4. Gorin, A. *Chem. Abstr.* **64** (1966) 8277 d and 11552 d.
5. Lindberg, B. and Swan, B. *Acta Chem. Scand.* **14** (1960) 1043.
6. Wilson, C. M. *Anal. Chem.* **31** (1959) 1199.
7. Zweig, G. and Whitaker, J. R., (Ed.), *Paper Chromatography and Electrophoresis*, Academic, New York and London 1967, Vol. I, p. 252.
8. McComb, E. A. and McCready, R. *Anal. Chem.* **24** (1952) 1630.
9. Neukom, H., Deuel, H., Heri, W. J. and Kuendig, W. *Helv. Chim. Acta* **43** (1960) 64.

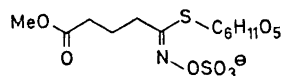
Received August 12, 1969.

## Glucosinolates in Some *Erysimum* Species

ROLF GMELIN\* and ANDERS KJÆR

Department of Organic Chemistry, Technical University of Denmark, Lyngby, Denmark

In 1957, we reported the presence of 3-carbomethoxypropylglucosinolate\*\* (I)



(I)

in seeds of the cruciferous species *Erysimum rupestre* DC., *E. ochroleucum* DC., and *E. pumilum* DC.<sup>1</sup> The seed materials employed were propagated in the Botanic Garden of the University of Copenhagen from small seed specimens donated and labelled by a German university botanic garden. Unfortunately, the seed-producing plants were not subjected to botanical control at the time of the cultivation.

In connection with other studies, the three seed samples were sown again in 1966, and the resulting plants were now kindly investigated botanically by Professor C. Favarger, Institut de Botanique, Université de Neuchâtel, who established the three originally studied "species" as identical and different from authentic specimens of *E. rupestre* DC., *E. ochroleucum* DC, and *E. pumilum* DC. According to Professor Favarger's diagnosis, the original studies were carried out on *Erysimum odoratum* Ehrh. which should therefore be regarded

\* On leave of absence from Freie Universität Berlin.

\*\* The trivial name "glucoerypestrin" was then proposed for this thioglucoside. Subsequent introduction, however, of a semisystematic "glucosinolate"-nomenclature has rendered the original and ambiguous nomenclature superfluous and undesirable (cf. Ref. 2).

as the first, and so far only source of the glucosinolate (I).\*

The present case may serve as a warning. Whenever sets of chemical and biological parameters (such as a botanical name) are linked together, care and caution must be exercised, also in the biological identification, in order to make the combined information meaningful.

Through the good offices of Professor Favarger, a small amount of seed of the authentic *Erysimum ochroleucum* DC. was put at our disposal and propagated in the Botanic Garden of the University of Copenhagen.\* Chromatographic analysis of the seed revealed its contents of two glucosinolates, identified as 3-methylthiopropyl-<sup>3</sup> and the corresponding 3-methylsulphinylpropyl-glucosinolate,<sup>4</sup> the latter with (*R*)-configuration around the sulphoxide grouping.<sup>5</sup> The identification was effected by enzymic hydrolysis to the corresponding isothiocyanates and conversion of the latter into crystalline thiourea derivatives which were compared with authentic specimens (see Experimental).

In conclusion, seeds of *Erysimum odoratum* Ehrh. contain (I) as the predominant glucosinolate. *E. ochroleucum* DC. does not contain (I) but possesses a distinctly different pattern as described above.

*Experimental.* Seeds of *Erysimum ochroleucum* DC. (25 g) were ground, defatted, and extracted with 70 % methanol. The extract residue was dissolved in water (500 ml) and passed through a column containing anionotropic alumina (Woelm, 100 g). The column was rinsed with water, and the glucosinolates were eluted with 1 % NaOH-solution; 25-ml fractions were collected. Fractions Nos. 5–8 were combined, diluted with an equal volume of citrate buffer (pH 6.8), and subjected to enzymic hydrolysis by the addition of a crude myrosinase solution and a trace of ascorbic acid. After standing for several hours at room temperature, the reaction mixture was extracted three times with ether.

The dried ether extract was mixed with a methanolic solution of ammonia. After an

\* Herbarium vouchers of plants grown from seed employed in the chemical work have been deposited in the Botanic Museum of the University of Copenhagen.

hour, the solution was taken to dryness, and the crystalline residue was recrystallized, first from ethyl acetate:petroleum ether, and then from water. The colourless crystals had m.p. 67–68° (uncorr.) alone, or in admixture with an authentic specimen of 1-(3-methylthiopropyl)thiourea.<sup>3</sup> The naturally derived and the synthetic thioureas exhibited coinciding infra-red spectra.

The aqueous phase from the ether extraction was extracted with three small portions of chloroform. To the dried solution was added a slight excess of aniline, the solution was set aside for 2 h at room temperature, and then concentrated to dryness. The crystalline residue was recrystallized, first from ether:petroleum ether, then from water to give colourless crystals, m.p. 134° (uncorr.), alone or mixed with an authentic specimen of (*R*)-1-(3-methylsulphinylpropyl)-3-phenyl-thiourea.<sup>6</sup>

The two specimens exhibited identical IR-spectra. The rotation,  $[\alpha]_D^{32} -45^\circ$  (*c* 1.5, EtOH), was numerically slightly lower than that previously reported ( $-54^\circ \pm 2^\circ$ ).<sup>6</sup>

The kind assistance of the *Botanic Garden of the University of Copenhagen* in the production of seed material for the present investigation is gratefully acknowledged. The authors are much indebted to Professor C. Favarger for his expert advice on the botanical identity of the various *Erysimum* species and for a generous seed sample of authentic *E. ochroleucum* DC. The support of the present investigation by *Statens Teknisk-Videnskabelige Fond* is gratefully acknowledged.

1. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **11** (1957) 577.
2. Ettliger, M. G. and Kjær, A. In Mabry, T. J., Alston, R. E. and Runeckles, V. C. *Recent Advances in Phytochemistry*, Appleton-Century-Crofts, New York 1968, Vol. 1, p. 100.
3. Kjær, A., Gmelin, R. and Larsen, I. *Acta Chem. Scand.* **9** (1955) 1143.
4. Kjær, A. *Fortschr. Chem. Org. Naturstoffe* **18** (1960) 122.
5. Cheung, K. K., Kjær, A. and Sim, G. A. *Chem. Commun.* **1965** 100.
6. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **10** (1956) 1100.

Received September 2, 1969.