GLUCOSINOLATES IN THE GENUS *ZILLA* (BRASSICACEAE)

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Traditionally, the genus *Zilla* Forsk. (Brassicaceae) is conceived as composed of the species *Z. macroptera* Cosson (A), *Z. biparmata* O. E. Schulz (B), *Z. spinosa* (L.) Prantl (C), and a variety of the latter: *Z. spinosa* v. *microcarpa* Dur. & Sch. (D) (1, 2). Some authors, however, regard the genus as monotypic, comprising two subspecies: *Z. spinosa* (L.) Prantl, subsp. *spinosa* (=*Z. myagroides* Forsk. =subsp. *costata* Maire et Weiller), and subsp. *macroptera* (Cosson) Maire et Weiller (=*Z. macroptera* Cosson=*Z. biparmata* O. E. Schultz) (cf. e.g. Ref. 3). All are spiny shrubs indigenous to the desert regions stretching from Morocco to Arabia and reputed, among the local populations, as useful remedies in the treatment of ailments *e.g.* kidney stones. We have studied the taxa (B), (C), and (D) for their content of glucosinolates (**1**), a characteristic class of ions ubiquitously present within the family Brassicaceae (4, 5).

Paper chromatography disclosed the presence of only one, and apparently the same, glucosinolate in extracts of seed-containing siliques of *Z. biparmata*, *Z. spinosa*, and *Z. spinosa* v. *microcarpa*. For each species, the glucosinolate fraction was isolated, an aliquot converted into a crystalline acetate (K-salt), and the remainder subjected to enzymic hydrolysis, followed by isolation and chromatographic purification of the sulfur-containing hydrolysis product.

In each case, chemical and spectroscopic evidence disclosed the identity of the glucosinolate as 2-hydroxy-3-butenylglucosinolate (**2**, and/or **3**), characterized as the crystalline penta-*O*-acetyl derivative (K-salt), as well as by its ability to undergo myrosinase-catalyzed hydrolysis to the corresponding isothiocyanate, spontaneously cyclizing to give a *dextrorotatory* 5-vinyl-oxazolidine-2-thione (**5**).[1] The optical rotation data for the isolated products are presented in table 1. In a previous paper, *Z. spinosa* was described as a species producing the levorotatory enantiomer (**4**) (goitrin) on enzymic hydrolysis (8).[2]

(*R*)-2-Hydroxy-3-butenylglucosinolate (**2**), affording the antithyroid (*S*) (−)-5-vinyl-oxazolidine-2-thione (**4**) (goitrin) subsequent to enzymatic hydrolysis,[3] is an established constituent

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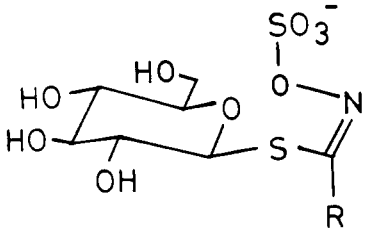
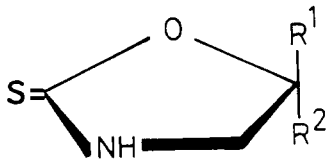
[1]The (*S*)-configuration (**4**) was established for the levorotatory enantiomer (goitrin) several years ago (6, 7).

[2]Though considerable variation in enantiomeric composition with strain, season, environment *etc.* is throughout conceivable, an experimental error in the sign of rotation of a preparation, brought to enantiomeric homogeneity by fractional crystallization, cannot be excluded.

[3]On the reasonable assumption that the configuration is retained during the cyclization.



1
2: R = (*R*) CH_2=CH-CH(OH)-CH_2-
3: R = (*S*) CH_2=CH-CH(OH)-CH_2-



4: R^1 = CH_2=CH-; R^2 = H
5: R^1 = H; R^2 = CH_2CH-

of many *Brassica* species (turnip, cabbage, kale, and rape) (9), whereas the epimeric (S)-glucosinolate (**3**), giving rise to (*R*) (+)-5-vinyloxazolidine-2-thione (**5**), has been encountered previously only in seeds of *Crambe abyssinica* (10). Reported rotation values of the penta-*O*-acetyl derivative of the (*R*)-glucosinolate (**2**), ($[\alpha]^{25}D$ −9.5°) (11), and the (*S*)-glucosinolate (**3**), ($[\alpha]^{25}D$ −14.8° and −15.1°) (10), together with those of the isolated and synthetic (*S*)-5-vinyloxazolidine-2-thione (**4**), $[\alpha]^{31}D$ −70.5° (MeOH) (9); $[\alpha]^{30}D$ −72.8° (MeOH) (synthetic) (12); $[\alpha]^{25}D$ −76.8° ($CHCl_3$) (10), and the (*R*)-enantiomer (**5**), $[\alpha]^{20}D$ +70.5° (MeOH) (synthetic) (12); $[\alpha]^{25}D$ +75.1° ($CHCl_3$) (10), justify the conclusion that the three *Z.* species all contain the epimeric (*S*)- and (*R*)-glucosinolates, (**2**) and (**3**), and in approximately the same ratio, *viz.* 9:1, based on the rotation values (table 1).

The identical glucosinolate patterns seem to support the monotypic treatment of the genus *Zilla*. Classification of *Z. biparmata* (=*Z. macroptera*) as a subspecies of *Z. spinosa* was also suggested recently on the basis of chromosomal similarities (13).

In rape plants, the biosynthesis of (**2**) proceeds from 2-amino-6-methylthiohexanoic acid (**6**), which, in its turn, derives from methionine (**7**) by two-fold homologization (14, 15); the side-chain hydroxylation seems to occur at a late stage along the synthetic route (14, 15). Assuming a similar pathway in *Zilla*, one is left with two possibilities:[4] (1) that two stereospecific hydroxylases are simultaneously operating, or (2) that the hy-

TABLE 1. Rotation data for penta-*O*-acetyl derivatives of glucosinolates (K-salts) and 5-vinyl-oxazolidine-2-thiones, from *Zilla* species.

Zilla species	$[\alpha]^{25}D$ for penta-*O*-acetyl derivatives in water (c, 2.4–2.6)	$[\alpha]D$ for 5-vinyl-oxazolidine-2-thiones
Z. biparmata	−14.6°	+61.5°[a]
Z. spinosa	−14.5°	+61.3°[b, c]
Z. spinosa v. *microcarpa*	−14.6°	+61.1°[b]

[a]At 26°; c, 2.5; MeOH.
[b]At 25°; c, 2.3–2.5; $CHCl_3$.
[c]Previously reported (7): −71°, at 31°; in $CHCl_3$ (*cf.* footnote 2).

[4]Epimerization of the glucosinolate fraction, or racemization of the 5-vinyl-oxazolidine-2-thiones, during the isolation and purification procedure seem highly unlikely in the present case.

droxylase in *Zilla* species is lacking stereospecificity. No choice between these possibilities can be made on the available evidence.

$$Me\text{-}S\text{-}[CH_2]_n\text{-}CH(NH_3^+)\text{-}CO_2^-$$

6: n=4
7: n=2

EXPERIMENTAL

PLANT MATERIAL.—Seed-containing siliques of all three *Zilla* species were collected in the Egyptian desert from plants authenticated by (the late) Professor V. Täckholm, Cairo University.

DETECTION AND EXTRACTION.—Paper chromatography of concentrated methanolic silique extracts was performed as previously reported (8). In each extract, one glucosinolate spot only could be seen.

Powdered and carbon tetrachloride-defatted, seed-containing siliques (200 g) of the various *Zilla* species were extracted with three 250 ml portions of 70% methanol. After removal of methanol *in vacuo*, water was added to a total volume of 2 liters, and the solutions were slowly passed through a column of anionotropic alumina (Woelm) (150 gm); the column was eluted with a 3% K_2SO_4-solution and the glucosinolate-containing fractions were taken to dryness and freed of most inorganic material by extraction into hot 90% ethanol.

CHARACTERIZATION AND ENZYMIC HYDROLYSIS.—One half of the glucosinolate fraction was subjected to acetylation in pyridine (5 ml) and acetic anhydride (5 ml) at 25° for 12 hours. The pentaacetates, recrystallized once from 95% ethanol, proved identical (uv, ir, pc) with an authentic specimen of the penta-*O*-acetyl derivative of the glucosinolate (**3**) isolated from *Crambe* seeds (10). Rotation: *cf.* table 1.

The second half of the glucosinolate fraction was dissolved in a citrate buffer (pH 6.8) and subjected to enzymic hydrolysis by the addition of a few drops of a myrosinase solution. After 6 hours, the solutions were extracted with chloroform, and the extracts were purified by chromatography on silica gel with benzene as the eluent and Grote's reagent as a diagnostic reagent for 5-vinyl oxazolidine-2-thiones. The still oily fractions containing the latter were again subjected to chromatography, now on silica gel (Merck 'Fertigsäule B'), with ethyl acetate as an eluent (6 ml/min) and uv-detection of the eluted oxazolidinethiones. The crystalline residues, mp ca. 50°, representing the total quantity of the latter, exhibited uv, ir (in solution), and ms data indistinguishable from those of an authentic specimen of (−)-5-vinyl-oxazolidine-2-thione (**4**); for rotations, *cf.* table 1.

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