Phytochemistry, 1970, Vol. 9, pp. 569 to 573. Pergamon Press. Printed in England.

GLUCOSINOLATES IN *MATTHIOLA FRUTICULOSA* AND RELATED SPECIES: A REINVESTIGATION

ROLF GMELIN* and ANDERS KJÆR

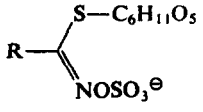
Department of Organic Chemistry, Technical University of Denmark, Lyngby, Denmark

(*Received* 2 *September* 1969)

Abstract—Seeds and plants of *Matthiola fruticulosa* (L.) Maire (*M. tristis* (L.) R. Br.) have been analysed for glucosinolates. The green parts contain 4-methylthio-3-butenylglucosinolate in addition to the corresponding 4-methylsulphinyl derivative; the seeds only the latter. Both glucosinolates are known from other crucifers, e.g. radish. The alleged occurrence in *M. fruticulosa* of methylglucosinolate, a widely distributed constituent of members of the Capparidaceae, could not be confirmed. In fact, methylglucosinolate seems to be strictly limited to the family Capparidaceae.

INTRODUCTION

CHEMICALLY the simplest glucosinolate encountered in nature has the methyl side-chain (I, R = CH_3). It was first found in various members of the Capparidaceae[1] and soon after isolated, as a crystalline potassium salt,† from the seeds of *Cleome spinosa* Jacq.[2] Subsequently, methylglucosinolate has been repeatedly encountered in species of the Capparidaceae[4, 5] whereas, with one exception, it has not been reported in any of the more than 400 species of the Cruciferae which have been examined so far, and which consistently contain glucosinolates (I) with side-chains other than methyl.[3, 6]



(I)

The one exception is the report by Matas[7] claiming the occurrence of methyl isothiocyanate in the distillate from macerates of leaves of the crucifer *Matthiola fruticulosa* (L.) Maire.‡ The exceptional nature of this finding prompted a reinvestigation of the plant, the results of which are presented below.

* On leave from Freie Universität Berlin.

† Originally,[1, 2] the name "glucocapparin" was proposed for this glucosinolate. Subsequent introduction of the systematic "glucosinolate" nomenclature[3] however, has rendered the original, ambiguous name superfluous and undesirable.

‡ The valid botanical name for the species; in the paper by Matas[7] the species is described under the synonym *M. tristis* (L.) R. Br.

[1] A. KJÆR, R. GMELIN and I. LARSEN, *Acta Chem. Scand.* **9**, 857 (1955).
[2] A. KJÆR and R. GMELIN, *Acta Chem. Scand.* **10**, 335 (1956).
[3] M. G. ETTLINGER and A. KJÆR, in *Recent Advances of Phytochemistry* (edited by T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES), Vol. 1, p. 58, Appleton–Century–Crofts, New York (1968).
[4] A. KJÆR and H. THOMSEN, *Acta Chem. Scand.* **16**, 783 (1962).
[5] A. KJÆR and H. THOMSEN, *Phytochem.* **2**, 29 (1963).
[6] A. KJÆR, *Fortschr. Chem. Org. Naturstoffe* **18**, 122 (1960).
[7] L. C. MATAS, *Farmacognosia, Madrid* **20**, 307 (1960).

RESULTS

Plant Material

Seeds and plants of *Matthiola fruticulosa* (L.) Maire were kindly collected in the wild in Spain by Prof. C. Gómez-Campo, Instituto Nacional de Investigaciones Agronomicas, Madrid. The fresh plant material was shipped by air in plastic bags and was air-dried in our laboratory before extraction.

Analysis and Isolation

(*a*) Seed. A 70 per cent methanol extract of ground, defatted seed was chromatographed on paper in two solvent systems: ((i) butanol:ethanol:water; and (ii) butanol:pyridine: water), as described in another paper.[8] The three authentic glucosinolates (I, R = CH_3; R = $CH_3SOCH{=}CH(CH_2)_2$; and R = $C_6H_5CH_2$) served as reference compounds. In both solvent systems, the first two compounds were indistinguishable from each other, and from the single glucosinolate spot observed in the seed extract.

The remaining solution was subjected to enzymic hydrolysis, and a sample of the resulting isothiocyanate fraction was chromatographed on silica gel plates with sulphoraphene (II) and cheirolin (III) as reference compounds (methyl isothiocyanate is too volatile for TLC chromatography). The *Matthiola* isothiocyanate was indistinguishable from sulphoraphene (II). The major part of the isothiocyanate was treated with methanolic ammonia and the resulting thiourea was subjected to TLC chromatography with methylthiourea and the thiourea derived from (II)* as reference compounds. The naturally derived thiourea was indistinguishable from the compound formed from (II) but clearly different from methylthiourea.

$CH_3SOCH{=}CH(CH_2)_2NCS$ (II) $\qquad$ $CH_3SO_2(CH_2)_3NCS$ (III)

In order to substantiate these preliminary conclusions, a larger seed sample (5 g) was extracted and the glucosinolate fraction was purified and subjected to enzymic hydrolysis. The resulting isothiocyanate was treated with aniline and thus converted into the corresponding phenylthiourea which proved identical with (−)-1-(4-methylsulphinyl-3-butenyl)-3-phenylthiourea[9] on critical comparison (mixed m.p., i.r., mass spectra).

(*b*) Green parts. A 70% methanol extract of the air-dried plant material (leaves, stems, and inflorescences) was chromatographed on paper as above. In addition to a slowly moving glucosinolate, apparently identical with that in the seed extract, a more lipophilic constituent was observed, migrating at about the same rate as benzylglucosinolate (I, R = $C_6H_5CH_2$). Enzymic hydrolysis, followed by conversion of the resulting isothiocyanates into thioureas, and chromatography of the latter—with 1-methyl- and sulphoraphene-thiourea as reference substances—supported the identity of the slowly moving thiourea derived from *Matthiola* plants with sulphoraphene-thiourea. *No trace of methylthiourea was discernible.*

* The thiourea was produced by brief reaction of sulphoraphene (II), obtained from radish seed,[9] with ammonia. It was noted earlier by us[10] that the reported thiourea (m.p. 219–220°, $[\alpha]_D^{15}$ − 72°)[9] does *not* represent sulphoraphene-thiourea, but rather a decomposition product, formed on prolonged standing of sulphoraphene in methanolic ammonia and possessing a much lower R_f value by paper chromatography than the authentic sulphoraphene-thiourea.

[8] R. GMELIN and A. KJÆR, *Phytochem.* **9**, 591 (1970).
[9] H. SCHMID and P. KARRER, *Helv. Chim. Acta* **31**, 1017 (1948).
[10] A. KJÆR and R. GMELIN, *Acta Chem. Scand.* **10**, 1100 (1956).

From its R_f values, stability properties, and co-occurrence with sulphoraphene-thiourea, it appears very likely that the more lipophilic thiourea represents 1-(4-methylthio-3-butenyl)-3-phenylthiourea (I, R = $CH_3SCH{=}CH(CH_2)_2$), derived from the sulphide isothiocyanate corresponding to sulphoraphene and recently established as the pungent enzymic hydrolysis product in disintegrated radish.[11]

Finally, steam distillation, a procedure employed by Matas,[7] was attempted. Enzymic hydrolysis of the glucosinolates, extracted from the whole, air-dried plants, followed by steam distillation and treatment of the distillate with ammonia, gave a thiourea which, on TLC chromatography in two solvent systems, proved clearly different from methylthiourea. On paper chromatography in water-saturated chloroform, decomposition of the thiourea apparently took place yielding one spot on the starting line and another possessing an R_{Ph} value (i.e. R_f value relative to that of 1-phenylthiourea) of 0·58, compared with 0·93 as the reported value for 1-(4-methylthio-3-butenyl)-thiourea.[11]

DISCUSSION

Our results clearly indicate that *seeds* of *Matthiola fruticulosa* (L.) Maire contains only one glucosinolate, furnishing sulphoraphene (II) on enzymic hydrolysis. This finding agrees with that of Matas,[7] and is hardly surprising in view of the previously reported chromatographic evidence of the presence of only one glucosinolate, possessing the properties of the radish seed glucosinolate,[9] in *M. incana*, *M. annua*, and *M. bicornis*.[12] In fact, the parent glucoside was isolated as a crystalline tetraacetate from the latter species.[13] Occasionally, however, seeds of *Mattiola* species contain traces of a much more lipophilic glucosinolate, most certainly representing the reduced form (I, R = $CH_3SCH{=}CH(CH_2)_2$) of the major thioglucoside. Enzymic hydrolysis, followed by steam distillation, may in such cases give rise to isothiocyanates, either the expected, rather labile mustard oil, or decomposition products thereof. Most likely, the volatile isothiocyanate from seed of *M. annua*, tentatively identified by us several years ago[14] as 4-methylthiobutyl isothiocyanate on basis of chromatographic data and a radish-like odour, in fact represented the unsaturated 4-methylthio-3-butenyl-isothiocyanate, the pungent principle of radish.[11] The rather labile character of the latter, as well as of the corresponding thiourea, easily gives rise to decomposition products, originally, but erroneously, reported by us as indicative of the presence of trace amounts of methyl- and isopropyl-glucosinolate in *M. annua*, and of the former in *M. fenestralis*.[15] This suggestion was later retracted.[1]

As apparent from the above results, *the green parts* of *M. fruticulosa* contain the sulphoraphene-producing glucosinolate and the corresponding sulphide as the only glucosinolates, the former representing the major constituent. Not even trace amounts of additional glucosinolates are observed, and we can provide no explanation for the reported claim[7] that methyl and isopropyl isothiocyanate are produced on enzymic hydrolysis other than artefact formation, as discussed above.* However, the alleged identification of the corresponding

* The reported production of 2-phenylethyl isothiocyanate in macerates of plants of *M. annua*[16] rests solely on meagre chromatographic evidence. It appears likely that the chromatographed thiourea in fact represented that derived from 4-methylthio-3-butenyl-isothiocyanate, possessing a rather similar R_f value.

[11] P. Friis and A. Kjær, *Acta Chem. Scand.* **20**, 698 (1968).
[12] O. E. Schultz and W. Wagner, *Z. Naturforsch.* **11b**, 73 (1956).
[13] O. E. Schultz and W. Wagner, *Arch. Pharm.* **288/60**, 525 (1955).
[14] A. Kjær and R. Gmelin, *Acta Chem. Scand.* **9**, 542 (1955).
[15] A. Kjær, J. Conti and I. Larsen, *Acta Chem. Scand.* **7**, 1276 (1953).
[16] P. Delaveau, *Bull. Soc. botan. France* **104**, 148 (1957).

crystalline thioureas by comparison with authentic specimens,[7] remains totally unintelligible to us. Hence, we consider the alleged existence of methylglucosinolate in *M. fruticulosa* as disproved.

Thus it remains an interesting fact that methylglucosinolate (I, R = CH_3), so typical for many genera and species of the Capparidaceae, is apparently absent from the more than 400 species of the related family Cruciferae examined so far, as well as from other glucosinolate-containing families.[3] Glucosinolates are biosynthesized from amino acids (cf. Ref. 3). The seeming inability of crucifers to utilize alanine, the required precursor for methylglucosinolate, is a puzzling fact which may deserve further attention.

EXPERIMENTAL

Glucosinolates were chromatographed on paper in the solvent systems: (A) *n*-BuOH:EtOH:H_2O (4:1:4), and (B) *n*-BuOH:pyridine:H_2O (6:4:3) as recently described.[8] R_B-R_f values signify the R_f-values relative to that of benzylglucosinolate (I, R=$C_6H_5CH_2$). TLC was performed on Kieselgel H (Merck) with the solvent systems: (A) as above, and (C) $CHCl_3$:MeOH (85:15). Melting points are uncorrected.

Chromatography and Enzymic Hydrolysis of Seed Glucosinolate

Paper chromatography of a 70% MeOH extract of ground, defatted seeds of *Matthiola fruticulosa* (L.) Maire gave one spot with R_B 0·21 in solvent system (A), 0·78 in system (B), the same as reference samples of the glucosinolates (I, R=CH_3) and (I, R=$CH_3SOCH{=}CH(CH_2)_2$).

The concentrated extract (from 1 g of seed) was diluted with citrate buffer (pH 6·4) and subjected to enzymic hydrolysis by the addition of a few drops of a myrosinase solution and a trace of ascorbic acid. The mixture was extracted thrice with $CHCl_3$. On TLC [solvent: $CHCl_3$:MeOH (95:5)], the isothiocyanate migrated at a rate corresponding to an R_f value of 0·38, the same as that of authentic sulphoraphene (II).

Cheirolin (III) had an R_f value of 0·52. (Spray reagent: $AgNO_3$ in ammonia). The remaining $CHCl_3$ extract was treated with an excess of methanolic NH_3. The resulting thiourea was subjected to TLC chromatography in system (A): R_f value 0·33, comparable to that of sulphoraphene-thiourea (0·33), and methylthiourea (0·54).

Phenylthiourea from seed of M. fruticulosa

A crude glucosinolate extract (50 ml), from 5 g of seeds of *M. fruticulosa*, was purified by ion exchange on anionotropic alumina (Woelm, 10 g); 0·2 N NaOH was employed for elution. The glucosinolate eluate was hydrolysed enzymically with myrosinase, the resulting isothiocyanate was extracted with $CHCl_3$ and the organic phase was washed with dil. Na_2CO_3 and H_2O. To the dried solution was added excess aniline, and the mixture was set aside for 4 hr at room temp. The solution was then concentrated and treated with Et_2O and light petroleum. The crystalline residue was recrystallized from ethyl acetate:light petroleum to give a colourless phenylthiourea (19 mg), m.p. 117°, alone or in admixture with authentic (−)-1-(4-methylsulphinyl-3-butenyl)-3-phenylthiourea.[9] The two specimens displayed identical i.r. spectra (in KBr) and mass spectra. Precise determination of the optical rotation was impossible due to lack of material but the observed rotation (*ca.* 80°) was negative, and of the same order of magnitude as reported for the authentic derivative {$[\alpha]_D^{18} - 105°$ ($CHCl_3$)}.[9]

Chromatography and Enzymic Hydrolysis of Plant Glucosinolates

The air-dried, green plants were disintegrated and extracted with 70% MeOH. Paper chromatography in solvent systems (A) and (B) revealed the presence of two spots with R_B values identical with those of (I, R=$CH_3SOCH{=}CH(CH_2)_2$) and (I, R=$C_6H_5CH_2$).

Enzymic hydrolysis in the usual fashion, and reaction of the isothiocyanate fraction with NH_3, gave two thioureas which, on TLC chromatography in solvent system (A), had R_f values of 0·42 and 0·70, the former identical with that of sulphoraphene-thiourea. Authentic methylthiourea possessed the R_f value 0·59.

Steam Distillation of Enzymically Hydrolysed Plant Extracts

Pulverized, air-dried plants of *M. fruticulosa* were extracted with 70% MeOH as above. The concentrated extract was subjected to enzymic hydrolysis, followed by steam distillation. The distillate was treated with ammonia and concentrated to dryness. TLC chromatography gave a thiourea with the R_f values: 0·49 [in system (C)] and 0·60 [in system (A)]. Reference samples of 4-methylthiobutylthiourea and methylthiourea had R_f values of 0·53 [in (C)] and 0·50 [in (A)], respectively.

On paper chromatography, with H_2O-saturated $CHCl_3$ as the mobile phase, two spots were observed, with R_{Ph} values (i.e. R_f values relative to that of phenylthiourea) of 0·0 and 0·58, attributable to decomposition products. Authentic methyl- and 4-methylthio-3-butenyl-thiourea migrates with R_{Ph} values of 0·03 and 0·93,[11] respectively, under these conditions.

Acknowledgement—The authors are much indebted to Prof. C. Gómez-Campo, Madrid, for his efficient assistance in providing us with seeds and fresh plants of *M. fruticulosa* (L.) Maire.

The support of the present investigation by *Statens Teknisk-Videnskabelige Fond* is gratefully acknowledged.