GC–MS analysis of volatile hydrolysis products from glucosinolates in *Farsetia aegyptia* var. *ovalis*

A. A. Al-Gendy and G. B. Lockwood*

School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester M13 9PL, UK

Received 16 November 2001
Revised 8 April 2002
Accepted 10 April 2002

ABSTRACT: Twenty-two volatile glucosinolate hydrolysis products from seeds and leaves of *Farsetia aegyptia* var. *ovalis* were identified and quantified using both natural autolysis and exogenous myrosinase, followed by GC–MS. The 22 isothiocyanate thiocyanate, epithioalkane nitrile and nitrile hydrolysis products identified can be rationalized, assuming 13 glucosinolates in the seeds and 12 in the leaves. Among the volatile hydrolysis products observed, those derived from allylglucosinolates and 3-methylsulphinylpropylglucosinolate (glucoiberin) were prominent.

KEYWORDS: *Farsetia aegyptia* var. *ovalis*; Cruciferae; glucosinolates; isothiocyanates; nitriles

Introduction

A number of species of the genus *Farsetia* have been used for treatment of rheumatism,¹ and glucosinolates have been reported in five species.² To date, 120 glucosinolates have been reported in genera from 16 plant families, and their volatile, pungent hydrolysis products are understood to possess a number of marked biological activities.² The earliest reported examination of *Farsetia* spp. for glucosinolate content was in 1955,³ when 4-(methylthio)butylglucosinolate was detected in *F. clypeata* R.Br.

More recently, glucosinolate contents of *F. aegyptia* and *F. ramosissima* were investigated in air dried leaves of both plants.⁴ *F. aegyptia* was shown to have at least six glucosinolates, but chiefly sinigrin (>80%). Without the addition of exogenous thioglucosidase enzyme (myrosinase), nitriles were the major hydrolysis products of glucosinolates. Normal hydrolysis of glucosinolates results in production of the isothiocyanate by the action of myrosinase. However, during hydrolysis, nitrile formation is favoured by low pH, but alternatively can be promoted instead of isothiocyanate formation by the presence of certain metal cations.⁵ The occurrence of thiocyanate products is considered to involve isomerase enzyme-mediated rearrangement of the isothiocyanate.⁵ Upon addition of extra enzyme, the corresponding isothiocyanates became the major products. Varying the pH from the natural level for the plant also considerably affected the ratios of glucosinolate hydrolysis products.

GC–MS analysis was used to identify the following glucosinolates from their hydrolysis products,⁴,⁵ sinigrin, 3-butenylglucosinolate (gluconapin), 2-phenylethylglucosinolate (gluconasturtiin), sec-butylglucosinolate, 3-methylthiopropylglucosinolate, benzylglucosinolate, and 4-methylthiobutylglucosinolate (glucoerucin).⁶

Materials and Methods

Plant Material

*Farsetia aegyptia* var. *ovalis* plants were collected from the Western Desert, near the Cairo–Alexandria desert road, Egypt, in March 1997. The plant material was identified at the Herbarium, University of Manchester Museum, Manchester M13 9PL, UK, and compared with authentic herbarium samples. A voucher sample, Manch Kk 1868, was deposited.

Preparation of Hydrolysis Products

Natural Autolysis⁷

Seeds and naturally dried leaves of *F. aegyptia* var. *ovalis* were analysed for the presence of glucosinolates by detection of their autolysis products. 250 mg crushed undefatted seeds or leaves were mixed with distilled water (100 ml), and left for autolysis overnight (17 h) at
27 ± 2 °C. Dichloromethane (20 ml) was added, the mixture was shaken for 30 min and separated by centrifugation for 5 min at 3500 rpm. The separated organic layer was dried over a small amount of anhydrous sodium sulphate, concentrated under nitrogen to 100 µl.

**Exogenous Hydrolysis**

Crushed undefatted plant material was mixed with water, myrosinase enzyme (1–2 units, Sigma; origin, *Sinapis alba*) and 2–5 mg L-ascorbic acid (Merck) and allowed to hydrolyse for 1 and 2 h, followed by extraction as above.

Leaf and seed material (1 g) were investigated for the presence of non-volatile hydrolysis products by homogenizing with boiling 70% methanol and refluxing for 10 min. After hot filtration, the hot marc was washed twice with 50% methanol. The methanol was removed from the combined filtrates under vacuum, and the concentrated aqueous extract defatted with dichloromethane (50 ml). Exogenous myrosinase hydrolysis of this aqueous extract was carried out as above. The concentrated dichloromethane extract (100 µl) was chromatographed by thin layer chromatography (silica gel G, 0.25 mm, dichloromethane:methanol 9:1 solvent system) and visualized with ammoniacal silver nitrate, followed by heating at 100 °C for 10 min. No spots were detected.

**Gas Liquid Chromatography (GLC) Analysis**

1–2 µl concentrated extract was analysed by GLC. A Hewlett-Packard 5890 GC fitted with a flame ionization detector was used for the analysis of volatile ionization autoxidation and exogenous myrosinase hydrolysis products. The column was a ZB-5 Zebron megabore capillary column, liquid phase 5% phenyl polysiloxane, 30 m × 0.53 mm, 5 µm film thickness. The results were recorded on a Hewlett-Packard 3392 A integrator. The oven temperature was programmed to 70 °C initially for 2 min, rising at 5 °C/min to 280 °C. Helium was the carrier gas and the flow rate was 2 ml/min. Mass spectrometer conditions were as follows: ionization current, 1A; temperature source, 150 °C; resolution, 1000; scan speed, 1 s/decade. Identification of hydrolysis products was based on sample retention time data, co-chromatography with available standards (but-3-enenitrile, allyl isothiocyanate, benzyl thiocyanate and benzyl isothiocyanate), and electron impact–mass spectrometry (EI–MS). Chemical ionization–mass spectrometry (CI–MS) was carried out using ammonia under the same operating conditions as for EI–MS.

**Results and Discussion**

Identification of Hydrolysis Products

GC–MS analysis provided the following spectra, which matched with previously published data,8–14 and this was used in combination with previously published retention data:

- **Allyl thiocyanate**, Rt. 2.3 min. EI–MS, m/z (% relative abundance); 38 (13%), 39 (76%), 41 (100%), 44 (5%), 45 (11%), 58 (5%), 72 (10%) and 99 (M+, 28%).
- **Allyl isothiocyanate**, Rt. 2.6 min. EI–MS, m/z (% relative abundance); 38 (19%), 39 (91%), 41 (100%), 44 (9%), 45 (13%), 58 (7%), 71 (11%), 72 (30%), 98 (11%) and 99 (M+, 90%). CI–MS, 117 (M+ + 18, 86%).
- **Sec-Butyl isothiocyanate**, Rt. 3.4 min. EI–MS, m/z (% relative abundance), 40 (79%), 41 (100%), 43 (24%), 44 (51), 55 (25%), 56 (64), 57 (59%), 58 (17%), 72 (11%), 86 (59%) and 115 (M+, 26%).
- **Isobutyl isothiocyanate**, Rt. 3.7 min. EI–MS, m/z (% relative abundance), 39 (49%), 41 (100%), 43 (63%), 55 (16%), 56 (30%), 57 (47%), 58 (7%), 72 (46%), 73 (38%) and 115 (M+, 27%). CI–MS, 116 (M+ + 1; 58%) and 133 (M+ + 18; 55%).
- **3-Butenyl isothiocyanate**, Rt. 4.2 min. EI–MS, m/z (% relative abundance), 41 (12%), 45 (36%), 46 (19%), 55 (22%), 64 (12%), 72 (100%), 85 (7%) and 113 (M+, 18%).
- **3,4-Epithiobutane nitrile**, Rt. 5.1 min. EI–MS, m/z (% relative abundance) 41 (24%), 45 (57%), 59 (91%), 66 (14%), 72 (56%) and 99 (M+, 100%). CI–MS, 100 (M+ + 1; 100%) and 117 (M+ + 18; 42%).

**Quantitative Estimation of Glucosinolate Hydrolysis Products**

Seven dilutions (0.06–0.78 µg/µl) of phenyl isothiocyanate in dichloromethane were used to construct a calibration curve. A plot of phenyl isothiocyanate concentration (µg/µl), against the peak area was linear, with \( r^2 \) value 0.993. The equation of the plot used to determine the level of glucosinolate hydrolysis products was:

\[
Y = 9214497.8(X) - 93831.9
\]
• 3-Methylbutyl isothiocyanate, Rt. 5.8 min. EI–MS, m/z (% relative abundance), 41 (100%), 43 (75%), 55 (28%), 57 (21%), 72 (38%), 73 (9%), 99 (9%), 114 (88%) and 129 (M+; 55%).

• 4-(Methylthio)butanenitrile, Rt. 6.9 min. EI–MS, m/z (% relative abundance), 41 (38%), 47 (25%), 54 (14%), 61 (100%), 68 (10%), 74 (9%) and 115 (M+; 53%). CI–MS, 116 (M+ + 1; 5%) and 133 (M+ + 18; 100%).

• Phenylacetonitrile, Rt. 7.2 min. EI–MS, m/z (% relative abundance), 39 (58%), 51 (49%), 65 (39%), 77 (24%), 89 (37%), 90 (35%), 91 (100%) 116 (29%) and 117 (M+; 72%), CI–MS, 135 (M+ + 18; 72%).

• 5-(Methylthio)pentanenitrile, Rt. 9.3 min. EI–MS, m/z (% relative abundance), 41 (21%), 47 (15%), 55 (22%), 61 (100%), 82 (37%), 87 (6%), 114 (4%) and 129 (M+, 40%). CI–MS, 130 (M+ + 1, 17%) and 147 (M+ + 18, 100%).

• Phenyl isothiocyanate, Rt. 9.7 min. EI–MS, m/z (% relative abundance), 50 (26%), 51 (48%), 58 (2%), 68 (11%), 77 (100%) and 135 (M+, 12%).

• 3-(Methylthio) propyl isothiocyanate, Rt. 12.3 min. EI–MS, m/z (% relative abundance), 41 (84%), 46 (21%), 47 (32%), 61 (44%), 72 (50%), 73 (21%), 86 (2%), 101 (100%) and 147 (M+, 12%). CI–MS, 148 (M+ + 1, 18%).

• Benzyl thiocyanate, Rt. 13.1 min. EI–MS, m/z (% relative abundance), 51 (31%), 65 (25%), 77 (10%), 91 (100%), 105 (8%), 121 (5%) and 149 (M+, 14%).

• Benzyl isothiocyanate, Rt. 13.8 min. EI–MS, m/z (% relative abundance), 58 (3%), 65 (21%), 72 (2%), 77 (2%), 91 (100%), 121 (6%) and 149 (M+, 53%).

• 4-(Methylsulphonyl) butanenitrile, Rt. 15.8 min. EI–MS, m/z (% relative abundance), 41 (49%), 56 (100%), 63 (13%), 64 (30%), 78 (8%), 84 (7%), 91 (5%), 131 (1%) and 147 (M+; 2%). CI–MS, 148 (M+ + 1; 41%) and 165 (M+ + 18;3%).

• 4-(Methylthio) butyl isothiocyanate, Rt. 16.1 min. EI–MS, m/z (% relative abundance), 41 (43%), 47 (33%), 55 (30%), 61 (100%), 72 (48%), 85 (36%), 100 (8%), 115 (66%), 146 (6%) and 161 (M+, 16%).

• 2-Phenylethyl isothiocyanate, Rt. 16.9 min. EI–MS, m/z (% relative abundance), 40 (22%), 51 (7%), 65 (10%), 72 (4%), 77 (10%), 91 (100%), 105 (12%) and 163 (M+; 21%).

• 3-(Methylsulphonyl)propyl isothiocyanate, Rt. 21.2 min. EI–MS, m/z (% relative abundance), 41 (66%), 61 (16%), 63 (30%), 72 (100%), 86 (15%), 100 (39%), 102 (9%), 116 (49%), 130 (13%), 148 (2%) and 163 (M+; 5%). CI–MS, 164 (M+ + 1, 100%), and 181 (M+ + 18, 48%).

• 3-(Methylsulphonyl)propyl isothiocyanate, Rt. 21.7 min. EI–MS, m/z (% relative abundance), 40 (99%), 41 (100%), 63 (9%), 72 (61%), 79 (16%), 80 (2%), 99 (37%), 121(7%) and 179 (M+; 25%). CI–MS, 197 (M+ + 18, 9%).

But-3-enenitrile cannot be adequately separated from the solvent peak in GC–MS, as reported previously. However, it was identified by the presence of characteristic fragments at m/z 41 and 67 for the allyl moiety and the molecular ion peak, respectively. Moreover, on GC, this compound showed complete matching (Rt.) with standard but-3-enenitrile. Pent-4-enenitrile has a poor spectrum because of incomplete separation from the adjacent peaks of but-3-enenitrile and allyl thiocyanate, due to close retention times. It was identified by the fragments at m/z 41, 55 and 81, which represent the allyl, butenyl and molecular ion peaks respectively.

Table 1 lists the individual glucosinolates tentatively identified from their hydrolysis products, and their levels. Thirteen glucosinolates were identified from the seeds and leaves of F. aegyptia. The possible occurrence of non-volatile indole or more polar glucosinolates was excluded by TLC screening. The glucosinolate hydrolysis products included isothiocyanates, thiocyanates, nitriles and epithioalkane nitriles. Allyl thiocyanates and benzyl thiocyanates were the only two thiocyanates detected, which were liberated from sinigrin and glucotropaeolin, respectively. These two glucosinolates were previously suggested to undergo Z–E isomerization of their aglycones by an isomerase. Sinigrin and glucobrassicin were the major compounds of the leaves and the seeds respectively. The previous investigations of F. aegyptia quantified only three glucosinolates, but identified traces of four others. Isobutyl, 3-methylbutyl and phenylglucosinolates were not previously identified in this plant, most probably due to their low concentration (0.01–0.03 µmol/g dry wt). Previous inability to detect the other glucosinolates, 4-(methylthio)butyl glucosinolate, 3-(methylsulphonyl)propyl glucosinate, 3-(methylsulphonyl)propyl glucosinolate and 4-(methylsulphonyl)butyl glucosinolate, may have been due to the use of short temperature programming, which was unsuitable for long-chain methylthio-, methylsulphinyl- and methylsulphonylglucosinolates. The presence of glucobrassicin in the seeds, and at relatively high levels, is reported here for the first time.

The variation of glucosinolate hydrolysis products and their levels between previous studies and the present work could be attributed to geographical and seasonal differences, environment, soil type, stress, genetic origin and plant part examined, or different experimental conditions.
The use of exogenous myrosinase hydrolysis for only 1 h caused dramatic reduction of glucosinolate levels, while using 0.5–1 units for 2 h hydrolysis provided good levels. Increasing the autolysis or exogenous hydrolysis time caused loss of minor glucosinolates, especially those that gave very volatile compounds.

Although levels of allyl thiocyanate and benzyl thiocyanate were detected in this work, 4-(methylthio)butyl thiocyanate was probably not detected due to very low levels of the parent glucosinolate. It is worth noticing the sharp difference in glucosinolate hydrolysis product levels, especially those derived from sinigrin obtained by natural autolysis and exogenous myrosinase from the leaves. Few published reports compare the levels of hydrolysis products derived from glucosinolates using both autolysis and exogenous myrosinase. In both one such report, and other work we carried out on whole plant material, levels varied enormously. This may be explained by the weak activity of the natural enzyme, due to either the lack of myrosin cells in the leaves or improper storage conditions. On the other hand, the relatively high levels of glucosinolate hydrolysis products obtained by natural autolysis of the seeds may indicate high levels of endogenous myrosinase.

Acknowledgements—The authors would like to thank the Egyptian Government and the ORS fund for sponsorship of A.A.A., and Dr Assem El-Shazly (Zagazig University, Zagazig, Egypt) for supplying the plant material.

References