Hydrolysis. A small amount of the isolate was heated in 1 N HCl at 100° for 1 hr. The products were then analysed with an automatic amino acid analyser. Hydrolysis was complete and equimolar amounts of Asp and NH_3 were detected.

Acknowledgements—We wish to thank Dr. Y. Niimura of Teikyo University for the ORD determination. This work was supported in part by the Special Coordination Funds for Promoting Science and Technology from the Science and Technology Agency of Japan and by a Grant-in-Aid for Special Project Research (No. 56122019) from the Ministry of Education Science and Culture.

REFERENCES

1. Tazawa, M. (1972) *Protoplasma* **75**, 427.
2. Kamiya, N. (1981) *Annu. Rev. Plant Physiol.* **32**, 205.
3. Chambers, R. W. and Carpenter, F. H. (1955) *J. Am. Chem. Soc.* **77**, 1522.
4. Bergmann, M. and Zervas, L. (1932) *Ber.* **65B**, 1192.
5. Ressler, C. (1960) *J. Am. Chem. Soc.* **82**, 1641.

Phytochemistry, Vol. 22, No. 10, pp. 2314–2315, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

FAILURE TO DETECT GLUCOSINOLATES IN *PLANTAGO* SPECIES

LONE MELCHIOR LARSEN, OLE OLSEN and HILMER SØRENSEN

Chemistry Department, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Copenhagen V, Denmark

(*Revised received* 16 *February* 1983)

Key Word Index—*Plantago major*; Plantaginaceae; glucosinolates; isothiocyanates; chemotaxonomy.

Abstract—Seeds and green parts of *Plantago* species have been examined for a supposed content of glucosinolates. Sensitive methods of quantitative glucosinolate analysis have been used but no glucosinolates could be detected. The significance of this investigation is briefly discussed in relation to chemotaxonomy and previously reported occurrence of isothiocyanates in extracts from *Plantago* species.

INTRODUCTION

Glucosinolates are well-known natural products co-occurring with myrosinases (thioglucoside glucohydrolase, EC 3.2.3.1) in all of the hitherto investigated plants belonging to Brassicaceae, Capparaceae and Resedaceae (ref. [1] and refs. cited therein). A restricted number of other plant families have been shown to contain glucosinolates mostly on the basis of degradation products thereof [2]. Furthermore, glucosinolates have been used in chemotaxonomy as compounds especially characteristic for the order Capparales [3].

Plantaginaceae is systematically remote from families in the order Capparales but, nevertheless, the possibilities of occurrence of glucosinolates in *Plantago* species have been discussed [3]. Plantaginaceae is either placed in its own order Plantaginales or incorporated into Tubiflorae, near the Scrophulariaceae.

An unidentified species of *Plantago* (plantain) has been reported to give a juice containing 4-methylsulfinylbut-3-enyl isothiocyanate (sulforaphene) [4]. The finding used as evidence for glucosinolates in *Plantago* has been questioned [2], but recently another report on products of autolysis from glucosinolates incorporated 8-week-old plants of *Plantago major* L. as the source of 5-vinyloxazolidine-2-thione (5 μg/g) and isopropyl isothiocyanate (1 μg/g) [5]. Such products of autolysis are traditionally used as evidence for presence of corresponding glucosinolates and myrosinases in the plants [1, 5]. Thus, the question concerning glucosinolates in Plantaginaceae is raised again.

The present work comprises investigations of *Plantago* species for their possible content of genuine glucosinolates. Recently developed sensitive methods of analysis based on techniques involving isolation of the intact glucosinolates have been used [1].

RESULTS AND DISCUSSION

Botanically well described *Plantago* species [6] were examined: green parts of *P. major* L. subsp. *major*, *P. major* subsp. *pleiosperma* Pilg. (rhamnose-type), *P. media* L., *P. lanceolata* L., *P. rugelii* Decne. and seeds of *P. major* L. subsp. *major*, *P. major* subsp. *pleiosperma* Pilg. (rhamnose-type), *P. major* subsp. *pleiosperma* Pilg. (glucose-type).

Extractions and isolation of fractions in which glucosinolates might be expected were performed by established methods including ion-exchange chromatography [7]. Careful analysis using HPLC [8], PC, HVE [1] and quantitative determination of glucose released by myrosinase treatment revealed no trace of glucosinolates whereas several other anions including carboxylates were present.

Freeze dried leaves of *P. major* subsp. *major* (20 g) and *P. major* subsp. *pleiosperma* (rhamnose-type) (8.7 g) afforded 14 mg and 42 mg, respectively, after prep. HVE at pH 1.9 of the fractions from the Ecteola columns [7].

Both extracts contained anions which moved like glucosinolates in HVE at pH 1.9, compounds which appeared as dark spots in 254 nm UV light and reacted with the silver nitrate reagent used for glucosinolate detection [7], but the light brown colour and the way it developed were quite different from a normal glucosinolate reaction. HPLC analysis of both extracts revealed the presence of two compounds with k'-values close to but different from those of authentic *N*-methoxyindol-3-ylmethylglucosinolate (neoglucobrassicin) [9] and indol-3-ylmethylglucosinolate (glucobrassicin) [8]. UV spectra showed an absorption maximum at 220 nm with a shoulder at 240 nm but no aromatic absorption which ruled out the possibility of any indolylmethylglucosinolate. Quantitative glucosinolate analysis based on treatment with isolated myrosinases [10] followed by determination of released glucose had a detection limit of 5 nmol glucosinolate as shown by use of authentic reference glucosinolates [10]. Based on these results it was concluded that the freeze dried leaves of *P. major* contained less than 1 nmol of glucosinolates per g. Seeds are normally a richer source of glucosinolates than green tissues, but due to shortage of greater amounts of authentic seed materials and clogging up of the ion-exchange columns by seed mucilage, no extractions of more than 500 mg portions of seeds were performed. Extracts were not examined for glucosinolate degradation products other than glucose since the genuine glucosinolates are now accessible to both qualitative and sensitive quantitative analyses [1].

In conclusion, intact glucosinolates were not detectable in the *Plantago* species investigated. However, absolute proof concerning the absence of plant constituents is very difficult to obtain, but our findings are in agreement with reported absence of myrosinase activity in *Plantago* species [2]. Thus, we do not believe that the Plantaginaceae can as yet be classified as a glucosinolate-containing family.

EXPERIMENTAL

Plant material. Seeds and leaves of the examined *Plantago* species were obtained by courtesy of Dr. Per Mølgaard, Royal Danish School of Pharmacy, who also divided *P. major* into the two subspecies and the pleiosperma subspecies into the two types containing either the rhamnose or glucose ester of caffeic acid [6].

General methods. These have been described elsewhere [7].

Isolation procedure and investigations for glucosinolates. Homogenization, extraction and ion-exchange chromatography were performed as previously described for isolation of intact glucosinolates from plant materials [7] except for use of a CM-Sephadex C-25 (1.6 × 30 cm) precolumn. Examinations for glucosinolates were performed by PC and HVE [1], and further purification of the extract by prep. HVE at pH 1.9 was followed by UV and HPLC [8] investigations and glucose determination after myrosinase treatment.

Glucose determination after myrosinase treatment. Aliquots of purified extracts in which glucosinolates might be expected were treated with myrosinases [10] for 2 hr and any glucose was determined by the Sigma glucose kit 115A, which contained hexokinase, glucose-6-phosphate dehydrogenase and a redox indicator system as described in the information sheet. Different forms of blind values were measured to correct for glucose contaminating the myrosinase preparation and the preparative isolated extracts.

Acknowledgements—We wish to thank Dr. Per Mølgaard, Royal Danish School of Pharmacy, Copenhagen, for his gift of the plant materials. Support from the Danish Agricultural and Veterinary Research Council is gratefully acknowledged.

REFERENCES

1. Olsen, O. and Sørensen, H. (1981) *J. Am. Oil Chem. Soc.* **58**, 857.
2. Ettlinger, M. and Kjaer, A. (1968) in *Recent Advances in Phytochemistry* (Mabry, T. J., Alston, R. E. and Runeckles, V. C., eds.) Vol. 1, p. 131. North-Holland, Amsterdam.
3. Rodman, J. R. (1981) in *Phytochemistry and Angiosperm Phylogeny* (Young, D. A. and Seigler, D. S., eds.) p. 43. Praeger, New York.
4. Prochazka, Z. (1959) *Naturwissenschaften* **46**, 426.
5. Cole, R. A. (1976) *Phytochemistry* **15**, 759.
6. Mølgaard, P., Skjøth, L. and Kaufmann, U. (1978) *Biochem. Syst. Ecol.* **8**, 277.
7. Olsen, O. and Sørensen, H. (1980) *Phytochemistry* **19**, 1783.
8. Helboe, P., Olsen, O. and Sørensen, H. (1980) *J. Chromatogr.* **197**, 199.
9. Christensen, B. W., Kjaer, A., Madsen, J.-Ø., Olsen, C. E., Olsen, O. and Sørensen, H. (1982) *Tetrahedron* **38**, 353.
10. Bille, N., Eggum, B. O., Jacobsen, I., Olsen, O. and Sørensen, H. (1983) *Z. Tierphysiol. Tierernaehr. Futtermittelkd.* **49** (in press).