Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3037–3038

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

THE CRUCIFEROUS PHYTOALEXINS BRASSININ AND CYCLOBRASSININ ARE INTERMEDIATES IN THE BIOSYNTHESIS OF BRASSILEXIN

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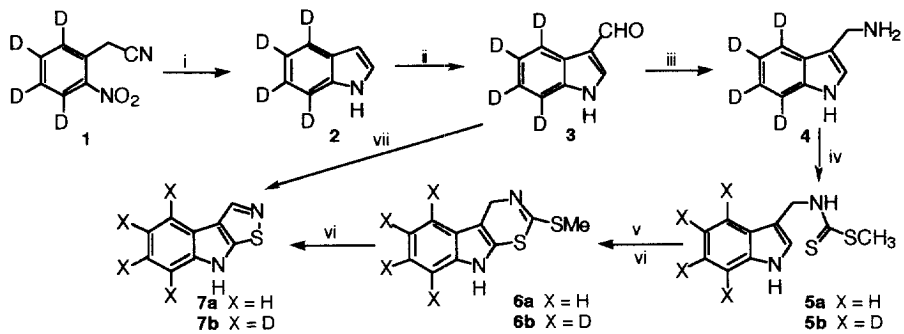
Received 24 June 1998; accepted 20 August 1998

Abstract: Following feeding experiments with the tetradeuterated cruciferous phytoalexins brassinin (**5b**) and cyclobrassinin (**6b**), leaves of *Brassica carinata* were elicited with the blackleg causing fungus *Phoma lingam* and incubated. Spectroscopic and HPLC analyses indicated that both brassinin (**5a**) and cyclobrassinin (**6a**) were incorporated into the cruciferous phytoalexin brassilexin (**7a**).

Plants have developed a variety of defense mechanisms against potential pathogens. One of such mechanisms, the accumulation of phytoalexins,[1] (antimicrobial compounds produced de novo by plants in response to pathogen attack and other forms of stress) at infection sites is considered an important factor in plant disease resistance.[2] For this reason, a large number of biological and chemical studies are directed to phytoalexins from plants of economic importance. Plants from the family *Cruciferae*, which comprises economically important vegetables (broccoli, cauliflower, cabbage, and turnip) and oilseed crops (canola, rapeseed, and mustard), produce an interesting array of phytoalexins (e.g., **5a**, **6a**, and **7a**), most of which are sulfur-containing indole derivatives.[3] Importantly, the resistance of diverse *Brassica* species to the devastating blackleg fungus [*Leptosphaeria maculans* (Desm.) Ces. et Not., asexual stage *Phoma lingam* (Tode ex Fr.) Desm] was reported to correlate with the accumulation of the phytoalexin brassilexin (**7a**).[4,5] Perhaps significantly, the accumulation of brassilexin (**7a**) was not observed in plants susceptible to the pathogen. We became interested in establishing the biosynthetic pathway to brassilexin in diverse *Brassica* species, as this knowledge is essential for an effective genetic manipulation of the phytoalexin pathway in cruciferous crops. Herein we wish to communicate work carried out to establish the possible biosynthetic precursors of brassilexin.



Scheme 1. Synthesis and biosynthesis of tetradeuterated phytoalexins; reagents and conditions: (i) H_2, 10% Pd/C, 93%;[6] (ii) DMF/$POCl_3$, 97%;[7] (iii) 1. NH_2OH/HCl, 2. Devarda's alloy, 70%; (iv) 1. Et_3N, CS_2, 2. MeI, 80%;[8] (v) pyridine/HBr/Br_2, 40%;[9] (vi) leaves of *Brassica carinata* elicited with *Phoma lingam*; (vii) 1. S_2Cl_2/AcOH, 2. NH_3/MeOH, 4%.[10]

No reports on brassilexin biosynthesis appear to have been published to date. Nonetheless, previous work[11,12] has indicated that brassinin (**5a**) via cyclobrassinin (**6a**), is a possible precursor of brassilexin (**7a**).This hypothesis was suggested by the in vitro oxidation of cyclobrassinin (**6a**) to brassilexin (**7a**),[12] as well as the transformation of **6a** into **7a** by the so-called "avirulent" blackleg fungus.[13] To investigate the brassilexin biosynthetic pathway, D_4-brassinin (**5b**), D_4-cyclobrassinin (**6b**), and D_4-brassilexin (**7b**) were synthesized as shown in Scheme 1.[14] Next, D_4-brassinin (**5b**) or D_4-cyclobrassinin (**6b**) were administered to

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00564-2

detached leaves of *B. carinata*.[15] Following incubation, the water droplets[15] from each leaf were collected, combined, and extracted with CH_2Cl_2. HPLC analysis[16] of the extracts indicated the presence of brassilexin and allowed its quantification by comparison with a standard calibration curve. Finally, the extracts were fractionated by HPLC to give pure brassilexin (**7a** and **7b**). The identity of the brassilexin samples were confirmed by ^1H NMR and HRMS-EI. The percentage of incorporation of D_4-brassinin and D_4-cyclobrassinin into brassilexin was determined to be *ca.* 2% and 0.5% respectively.[17]

These results indicate that both brassinin (**5a** or **5b**) and cyclobrassinin (**6a** or **6b**) are biosynthetic precursors of brassilexin (**7a** or **7b**) (Scheme 1). The lower incorporation of **6b** relative to **5b** is somewhat surprising, since in *B. carinata*, as in other plant species,[11] cyclobrassinin (**6a** or **6b**) is expected to be derived from brassinin (**5a** or **5b**) and therefore to be a closer biosynthetic precursor of **7a** than **5a**. Nonetheless, factors such as the lower solubility and higher phytotoxicity of **6a** relative to **5a** could account for a lower incorporation. In this connection it is worthy to note that, in uptake experiments cyclobrassinin was more phytotoxic to petiole and leaf tissue of *B. carinata* than brassinin; however, both compounds were phytotoxic, causing wilting and necrosis in petioles and leaf veins at concentrations > 3×10^{-4} M. We suspect that this damage to tissues may affect the biosynthesis of brassilexin and account for their low incorporation from the exogenous precursor pool.

Acknowledgments

Support for the authors' work was obtained in part from: the Natural Sciences and Engineering Research Council of Canada (in the form of a NRC-NSERC Research Partnership grant to M.S.C.P.), and the University of Saskatchewan. We would like to thank Ken Thoms, Department of Chemistry, for mass spectroscopic determinations

References and Notes

1. Brooks, C. J.; Watson, D. G. *Nat. Prod. Rep.* **1991**, 367, and references therein.
2. (a) Dixon, R. A. *Biol. Rev.* **1986**, *61*, 239; (b) Dixon, R. A.; Harrison, M. J.; Lamb, C. J. *Annual Rev. Plant Phytopathol.* **1994**, *32*, 479; (c) Kuc, J. *Annual Rev. Phytopathol.* **1995**, *33*, 275; (d) Smith, C. J. *New Phytol.* **1996**, *132*, 1.
3. For a recent review on brassica phytoalexins: Pedras, M. S. C.; Khan, A. Q.; Taylor, J. L. In *Phytochemicals for Pest Control*; Hedin, P. A.; Hollingworth, R. M.; Masler, E. P.; Miyamoto, J.; Thompson, D. G. Eds.; ACS Symposium Series 658, 1997; pp 155-166.
4. First isolation: Devys, M.; Barbier, M.; Loiselet, I.; Rouxel, T.; Sarniguet, A.; Kollmann, A.; Bousquet, J. *Tetrahedron Lett.*, **1988**, *29*, 6447.
5. Rouxel, T.; Kollmann, A.; Boulidard, L.; Mithen, R. *Planta*, **1991**, *184*, 271.
6. Van Den Berg, E. M. M.; Baldew, A. U.; de Goede, A. T. J. W.; Raap, J. ; Lugtenburg, J. *Rec. Trav. Chim. Pays. Bas*, **1988**, 107, 73.
7. Smith, G. F. *J. Chem Soc.*, **1954**, 3842.
8. Pedras, M. S. C.; Taylor, J. L. *J. Nat. Prod.* **1993**, *56*, 731.
9. Takasugi, M.; Monde, K.; Katsui, N.; Shirata, A. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 285.
10. Devys, M.; Barbier, M. *Organic Preparation & Procedure, International*, **1993**, *25*, 344.
11. Monde, K.; Takasugi, M.; Ohnishi, T. *J. Am. Chem. Soc.*, **1994**, *116*, 6650.
12. Devys, M.; Barbier, M. *Z. Naturforsch.*, **1992**, *47c*, 318.
13. Pedras, M. S. C.; Okanga, F. I. *Chem. Commun.*, 1998, 67.
14. All compounds gave satisfactory spectroscopic data; MS analysis indicated 99% incorporation of deuterium (similar to starting material **1**) for synthetic compounds **2-4**, **5b**, **6b**, and **7b**.
15. Typical feeding experiments: Petiolated leaves from 4-week-old plants were cut and immediately placed in Falcon tubes containing 1 mL of labeled D_4-brassinin (3×10^{-4} M in 10% MeOH and 0.05% Tween 80 in H_2O) or D_4-cyclobrassinin (2×10^{-4} M, 10% MeOH and 0.1% Tween 80 in H_2O). Following uptake of the solution, the leaves were placed upper side down in a plastic washtub containing premoistened paper towels. A droplet (10 µl) of a spore suspension of *P. lingam* (10^8 spores/mL) was applied to punctured areas on the underside of each leaf (20 to 100 droplets per leaf). The leaves were incubated at room temperature for 48 h.
16. HPLC analysis: photodiode array detection, carried out as reported in Pedras, M. S. C.; Khan, A. Q. *J. Agric. Food Chem.* **1996**, *44*, 3403. HPLC retention times (r_t, min): brassilexin r_t = 12.3; cyclobrassinin r_t = 24.8; brassinin r_t = 18.8.
17. The percentage of incorporation of D_4-brassinin (2.04 ± 0.09) and D_4-cyclobrassinin (0.34 ± 0.05) into brassilexin was calculated from the ratios of the areas of the (M^+) and the ($M^+ + 4$) peaks obtained for each sample; these ratios were compared with standard mixtures of brassinin/D_4-brassinin and cyclobrassinin/D_4-cyclobrassinin.