

In vitro Demonstration of a Biosynthetic Sequence for the Cruciferae Phytoalexins

Michel Devys and Michel Barbier

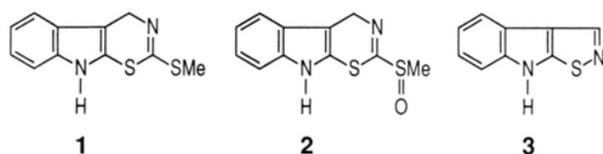
Institut de Chimie des Substances Naturelles, CNRS, Avenue de la Terrasse, F-91198 Gif-sur-Yvette, Cedex, France

Z. Naturforsch. **47c**, 318–319 (1992); received September 2/November 25, 1991

Cyclobrassinin Monosulphoxide, Brassilexin, Phytoalexins, Cruciferae

Periodate-induced degradation of cyclobrassinin monosulphoxide gives brassilexin in 40% yield. The sequence: cyclobrassinin to brassilexin through cyclobrassinin sulphoxide is now demonstrated for the group of phytoalexins found in the Cruciferae.

Previous publications reported on the isolation of a series of indole-derived phytoalexins from vegetables belonging to the Cruciferae family [1–5]. These substances are particularly efficient against the fungus *Phoma lingam* (Tode ex. Schw.) (*Leptosphaeria maculans* (Desm. Ces. et de Not.)) responsible for the blackleg disease of crucifers (for review see [6]). Among these phytoalexins, brassilexin (**3**) appears to exhibit the strongest biological activity. In spite of an obvious origin starting from tryptophan, the biosynthetic pathway leading to this series of products is still a matter of speculation. The presence of an intense metabolism of tryptophan among crucifers was demonstrated by the isolation of indole-3-carboxaldehyde from the cabbage *Brassica oleracea* [7] in a fairly large amount. The isolation of cyclobrassinin monosulphoxide (**2**) [5] leads to the hypothesis that this product is an intermediate in the biosynthesis of brassilexin (**3**) starting from **1**. To clarify this assumption, *in vitro* assays were carried out, and



1, Cyclobrassinin; **2**, cyclobrassinin monosulphoxide; **3**, brassilexin.

Reprint requests to Dr. M. Barbier.

Verlag der Zeitschrift für Naturforschung,
D-W-7400 Tübingen
0939–5075/92/0300–0318 \$ 01.30/0

previously the oxidative ring contraction of cyclobrassinin (**1**) into brassilexin (**3**) could be established [8]. In this series of assays, cyclobrassinin monosulphoxide (**2**) could not be observed among the secondary oxidation products. In the present publication, we report on the synthesis of cyclobrassinin monosulphoxide (**2**), and on its periodate-induced ring contraction to brassilexin (**3**), thereby bringing the complete *in vitro* demonstration of this hypothesis.

Cyclobrassinin monosulphoxide (**2**) was synthesized from cyclobrassinin (**1**) through oxidation by *m*-chloroperbenzoic acid. Isolation from the reaction mixture was carried out by preparative SiO₂-TLC, and identification was done by direct comparison of the physicochemical data with an authentic sample of **2** [5] (*R*_F, m.p., MS, high resolution MS, ¹H NMR, yield 80% from **1**). The periodate oxidation of cyclobrassinin monosulphoxide (**2**) was carried out in methanol–water as reported [8]. Brassilexin (**3**) was isolated with a 60% yield and obtained pure by repeated SiO₂-TLC (pentane–ethyl acetate 7:3, *R*_F 0.60; CH₂Cl₂ *R*_F 0.30, UV observation). Analytical grade of brassilexin was prepared by a further filtration on a small Al₂O₃ column (the final yield from **2** was 40%). The recovered **3** (m.p. 164–168 °C) was identified by direct comparison with an authentic sample either a natural [4] or a synthetic one [9] (MS, high resolution MS, ¹H NMR).

As the yield of the reaction carried out with cyclobrassinin monosulphoxide (**2**) is better than observed with cyclobrassinin (**1**), it appears that **2** is a more direct precursor of brassilexin (**3**) (at least *in vitro*). Accordingly, it may now be assumed that cyclobrassinin monosulphoxide (**2**) is a possible biological link between **1** and **3**. The biosynthetic pathway can be related to the action of cellular oxidases, a mechanism enhanced by liberation of these enzymes during the attack by the fungus. Oxidations result first in the formation of the sulphoxide (**2**), then the ring contraction is produced yielding **3** through a mechanism which was previously suggested [8]. The possible role of the corresponding disulphoxide of **1** cannot be excluded, but however this product could never be detected as yet from natural or synthetic sources.

Recently, growth inhibitions on human cancer cell cultures were determined for the indole sulphur-containing phytoalexins **1** and **3** [10].



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition “no derivative works”). This is to allow reuse in the area of future scientific usage.

Acknowledgements

Due thanks are expressed to Drs. B. C. Das, C. Girard and J. P. Dupuis for EI/MS determinations, to Mrs. C. Pasquier and C. Fontaine for the

^1H NMR carried out at Gif-sur-Yvette. The high resolution MS were carried out at the Centre de Mesures Physiques de l'Ouest, Rennes University.

- [1] M. Takasugi, N. Katsui, and A. Shirata, *J. Chem. Soc.* **1986**, 1077.
- [2] M. Takasugi, K. Monde, N. Katsui, and A. Shirata, *Chem. Lett.* **1987**, 1631.
- [3] M. Takasugi, K. Monde, N. Katsui, and A. Shirata, *Bull. Chem. Soc. Jap.* **61**, 285 (1988).
- [4] M. Devys, M. Barbier, I. Loiselet, A. Kollmann, and J. F. Bousquet, *Tetrahedron Lett.* **29**, 6447 (1988).
- [5] M. Devys, M. Barbier, A. Kollmann, T. Rouxel, and J. F. Bousquet, *Phytochemistry* **29**, 1087 (1990).
- [6] R. L. Gabrielson, *Seed Sci. Technol.* **11**, 749 (1983).
- [7] M. Devys and M. Barbier, *Phytochemistry* **30**, 389 (1990).
- [8] M. Devys and M. Barbier, *J. Chem. Soc. Perkin Trans. 1* **1990**, 2856.
- [9] M. Devys and M. Barbier, *Synthesis* **1990**, 214.
- [10] C. Tempête, M. Devys, and M. Barbier, *Z. Naturforsch.* **46c**, 706 (1991).