In vitro Demonstration of a Biosynthetic Sequence for the Cruciferae Phytoalexins

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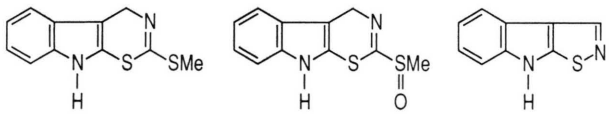
Periodate-induced degradation of cyclobrassinin monosulphoxide gives brassilexin in 40% yield. The sequence: cyclobrassinin to brassilexin through cyclobrassinin sulphoxide is now demonstrated for the group of phytoalexins found in the Cruciferae.

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 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.



1 **2** **3**

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

Cyclobrassinin monosulphoxide (**2**) was synthesized from cyclobrassinin (**1**) through oxidation by *m*-chloroperbenzoic acid. Isolation from the reaction mixture was carried out by preparative SiO_2-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 SiO_2-TLC (pentane–ethyl acetate 7:3, R_f 0.60; CH_2Cl_2 R_f 0.30, UV observation). Analytical grade of brassilexin was prepared by a further filtration on a small Al_2O_3 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].

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