3′-Hydroxylation of 4′-Methoxyisoflavones by *Fusarium oxysporum* f. *lycopersici*

K. Mackenbrock and W. Barz

Lehrstuhl für Biochemie der Pflanzen, Westfälische Wilhelms-Universität, Hindenburgplatz 55, D-4400 Münster

Z. Naturforsch. **38 c,** 708–710 (1983); received June 9, 1983

Hydroxilation, Isoflavones, Fusarium, Metabolism

3′-Hydroxylation of isoflavones by *Fusarium oxysporum* f. *lycopersici* mainly proceeds with 4′-methoxy-7-hydroxyderivatives; this reaction is used *for quantitative conversion* of ^{14}C-labelled isoflavones.

Introduction

Due to their fungitoxic properties isoflavones and pterocarpans represent interesting substrates for fungal metabolism [1]. Among the various initial reactions of isoflavone metabolism by *Fusarium* fungi [2–4] 3′-hydroxilation as recently found [3] with the isoflavones biochanin A (Fig. 2, **I a**) and formononetin (**I b**) is of special interest. Hydroxylation adjacent to an existing methoxyl group has only rarely been found and warrants further investigations.

Using a strain of *Fusarium oxysporum* f. *lycopersici* we report some results on the substrate specificity of isoflavone 3′-hydroxylation, the quantitative extent of this reaction and on its use for obtaining ^{14}C-labelled isoflavones.

Results and Discussion

In standard incubation assays with mycelial preparations of *F. oxysporum* f. *lycopersici* isoflavone (10^{-4} M) metabolism was quantitatively followed in aliquots by either TLC (S1) with subsequent scanning in case of ^{14}C-labelled substrates, or by HPLC. Thus, the efficient conversion of Ia and Ib into pratensein (**III a**) and calycosin (**III b**), respectively, could be demonstrated. As shown in Fig. 1 biochanin A 3′-hydroxylation proceeds much more rapidly than formononetin metabolism. Quantitative accumulation of pratensein is reached after appr. 13 h whereas maximum formation of **III b** requires some 28 h. Under our experimental conditions **III b** appeared to be an endproduct whereas **III a** is slowly further degraded. Attempts to isolate any catabolites of **III a** have so far failed.

0341-0382/83/0900-0708 $ 01.30/0

Similar experiments with genistein (**II a**) and daidzein (**II b**) revealed that only **II a** could be 3′-hydroxilated to a very low extent (formation of orobol (**IV a**) ~ 10% within 55 h) with the rest of the substrate being left unreacted. Daidzein as well as 5,7,4′-trimethoxyisoflavone and 6,7-dihydroxy-4′-

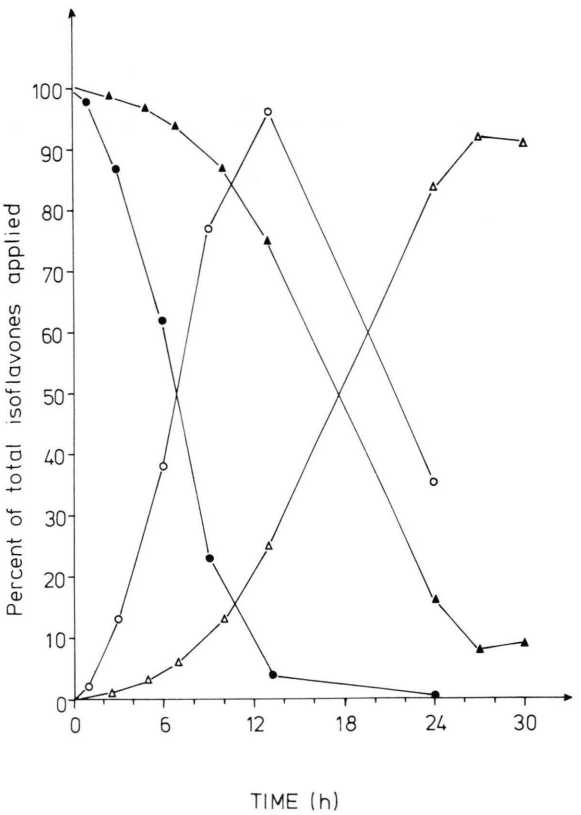

Fig. 1. Conversion of biochanin A (●—●) into pratensein (○—○) and of formononetin (▲—▲) into calycosin (△—△) by *Fusarium oxysporum* f. *lycopersici*. Isoflavone transformation was quantitatively followed by measuring both substrate and product after TLC or HPLC separation.

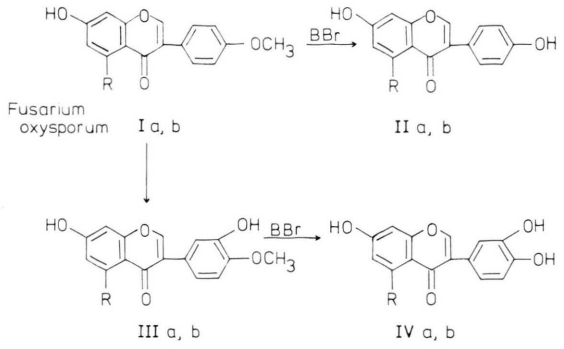

Fig. 2. The isoflavones biochanin A (**Ia**) or formononetin (**Ib**) may quantitatively be converted into a series of hydroxyderivatives by a combination of fungal (3′-hydroxilation by *F. oxysporum* f. *lycopersici*) and chemical reactions. This scheme is used to prepare ^{14}C-labelled isoflavones **IIa–IVb** from ^{14}C-**Ia** and -**Ib**.

Ia R = –OH: Biochanin A
Ib R = –H: Formononetin
IIa R = –OH: Genistein
IIb R = –H: Daidzein

IIIa R = –OH: Pratensein
IIIb R = –H: Calycosin
IVa R = –OH: Orobol
IVb R = –H: 7,3′,4′-Trihydroxyisoflavone

methoxyisoflavone (texasin) were not 3′-hydroxilated by *F. oxysporum* f. *lycopersici*.

The enzyme activity of this *Fusarium* strain for isoflavone 3′-hydroxilation which appears to be rather specific for a 7-hydroxy-4′-methoxyisoflavone skeleton, did not readily respond as an inducible enzyme system. Preincubation of mycelial preparations with Ia or Ib for up to 27 h did not significantly shorten the lag phase of 3′-hydroxilation nor increase the velocity of isoflavone metabolism.

The quantitative 3′-hydroxilation of both biochanin A and formononetin (Fig. 1) has been used as one step in the preparation of ^{14}C-labelled isoflavones with a 3′,4′-disubstituted B-ring. [^{14}C]Biochanin A or -formononetin accessible in rather large amounts by application of ^{14}C-labelled acetate, phenylalanine or cinnamic acid to roots of chick pea plants (*Cicer arietinum* L.) [5] may readily be converted by *F. oxysporum* to pratensein or calycosin in 100 mg quantities. Subsequent O-demethylation to **IVa** and **IVb** with BBr_3 also proceeds quantitatively [6]. As shown in Fig. 2 a combination of fungal and chemical reactions leads from **Ia** or **Ib** to a variety of other isoflavones (**IIa–IVb**) which may thus be synthesized in position-specific labelled form in excellent yield.

Experimental

Fungus

Fusarium oxysporum Schlecht ex Fr. f. *lycopersici* (Sacc.) Syn. u. Hans. (Centraalbureau voor Schimmelcultures, CBS 163.30) was stored and grown as previously described [3].

Standard assay

Degradation experiments with isoflavones (10^{-4} M), isolation of products and incubation conditions were carried out according to earlier reports [2, 3].

Large scale incubations

Fungal mycelium (80 g) and 100 mg isoflavone were incubated in 2 l potassium phosphate buffer (pH 7.5, 0.05 M) until maximum production of product (monitored by TLC or HPLC). Product was isolated by ether extraction of the medium and purified by chromatographic techniques. Yield: 80–90%.

Demethylation of isoflavones

Isoflavones were O-demethylated with BBr_3 in dry methylenechloride according to [6]. Hydroxyisoflavones can be recovered quantitatively. All products were characterized as previously reported [3, 4].

Chromatography

TLC on silica gel was performed with the solvent S_1: dichloromethane/methanol = 15:1. Isolation of isoflavones by Lobar column chromatography was by previous methods [4]. The HPLC separation [4] was carried out with the gradient of 20% B to 60% B in (A + B) in 35 min with A being 3% acetic acid and B acetonitril.

^{14}C-*Labelled isoflavones*

^{14}C-labelled samples of biochanin A and formononetin were from previous studies [4, 5]. Detection and measurement of ^{14}C-substrates have been described [3]. All other isoflavones were from the institute's collection.

Acknowledgement

Financial support by Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie is gratefully acknowledged.

[1] H. D. Van Etten, D. E. Matthews, and D. A. Smith, in: J. A. Bailey, J. W. Mansfield, eds. Phytoalexins chapter 6, Blackie, Glasgow/London 1982.
[2] U. Willeke and W. Barz, Z. Naturforsch. **37c,** 861 (1982).
[3] U. Willeke and W. Barz, Arch. Microbiol. **132,** 266 (1982).
[4] K.-M. Weltring, K. Mackenbrock, and W. Barz, Z. Naturforsch. **37c,** 570 (1982).
[5] W. Barz and H. Grisebach, Z. Naturforsch. **22b,** 627 (1967).
[6] J. F. W. McOmie, M. L. Watts, and D. E. West, Tetrahedron **24,** 2289–2292 (1968).