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DEGRADATION OF THE PHYTOALEXIN MEDICARPIN BY *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

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Key Word Index—*Fusarium oxysporum*; Hyphomycetes; pterocarpan; phytoalexin; medicarpin; degradation; 3-phenylcoumarin; isoflav-3-ene.

Abstract—*Fusarium oxysporum* f. sp. *lycopersici* completely degrades the pterocarpan phytoalexin medicarpin. Three intermediates of the catabolic pathway were elucidated by spectroscopic techniques as 7,2′-dihydroxy-4′-methoxyisoflavan (vestitol), 7,2′-dihydroxy-4′-methoxyisoflav-3-ene and 7,2′-dihydroxy-4′-methoxyisoflav-3-en-2-one.

INTRODUCTION

As part of our studies on the degradation of fungitoxic isoflavones and pterocarpans by *Fusarium* fungi [1–6], the catabolism of the phytoalexin [7] medicarpin (**1**) has been studied in *F. oxysporum* f. sp. *lycopersici* [1]. Previous reports on the fungal conversion of **1** dealt with initial reactions such as *O*-demethylation at C-9 [4, 8], hydroxylation at either C-4 or C-6a [8] and cleavage of the benzyl–phenylether bond leading to a 2′-hydroxyisoflavan [4, 8, 9]. Furthermore, the oxidative formation of both an isoflavanone [10] or a 1a-hydroxydienone [10] has also been described. All previous publications failed to demonstrate the subsequent degradation of the initial metabolites formed from **1**.

Our studies were aimed at elucidating a catabolic scheme for the isoflavan carbon skeleton of **1** and we now report on several new intermediates in medicarpin degradation obtained with *F. oxysporum* f. sp. *lycopersici*.

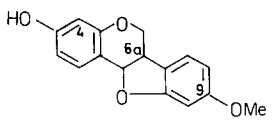
RESULTS AND DISCUSSION

During screening experiments of *Fusarium* fungi [1] for the disintegration of the carbon skeleton of **1**, mycelial suspensions of *F. oxysporum* f. sp. *lycopersici* were shown to completely degrade **1** (10^{-4} M) within 20 hr to non-aromatic compounds. The major phenolic metabolites from 108 mg **1** which transiently accumulated between 12–14 hr of incubation were extracted with ether and purified by TLC (S_2, main band R_f 0.52). Subsequent gel filtration over Sephadex LH 20 with methanol resulted in the separate elution of three compounds (**4**, **2** and **3** according to sequence of elution) which were individually purified further by TLC (S_1) and crystallization. Based on ^{1}H NMR and MS data (Experimental) as well as on cochromatography, **2** was readily identified as the known 7,2′-dihydroxy-4′-methoxyisoflavan (vestitol) [7]. Formation of this compound represents the well established reductive cleavage of the dihydrofuran ring of **1** [4, 7, 8, 9].

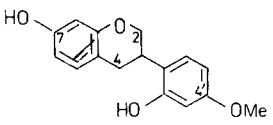
Compund **3** (R_f 0.34, S_1) was 7,2′-dihydroxy-4′-methoxyisoflav-3-ene as proven by the NMR and MS data. The strong $[M-1]^+$ peak (m/z 269 (56%)) indicates the formation of an isoflavylium ion [11] and, furthermore, an isoflav-2-ene structure can be excluded because the characteristic ring A-fragments of a Retro-Diels–Alder pattern (m/z 123 and 122) are missing. The ^{1}H NMR data clearly demonstrate the aromatic protons of the two rings of **3** in addition to the proton at C-4 (δ6.53) and the signal (δ4.95) of the methylene group at C-2 [11]. This again excludes a benzylic CH_2-group at C-4 (at δ3.5) as expected for an isoflav-2-ene [12].

The yellow compound **4** (TLC, S_1, R_f 0.37) was identified as 7,2′-dihydroxy-4′-methoxyisoflav-3-en-2-one from the ^{1}H NMR and mass spectra [13, 14]. The proton at C-4 (δ7.83) is very characteristic and excludes the possibility that **4** could be a coumestan derivative.

Since the allylic methylene group in **3** can readily be oxidized by mild chemical procedures and by molecular oxygen [17], control experiments with **3** were carried out to confirm that the formation of **4** had resulted from fungal enzymes. Thus, when samples of the isoflav-3-ene **3** were shaken without fungal cells in buffer under air for 15 hr and worked up in the usual way not even trace amounts of **4** (TLC) could be detected.



1



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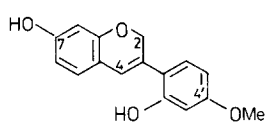

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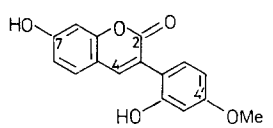

4

The formation of **3** and **4** from **1** through the isoflavan **2** as postulated by our studies has hitherto not been reported for microbial systems though such reactions have been discussed in connection with coumestan biosynthesis in plants [13, 15, 18]. The occurrence of both isoflav-3-enes [11, 16] and 3-phenylcoumarins [14, 19] has so far been reported for only a few plants. Dehydrogenation of **2** yielding **3** with subsequent oxidation to **4** followed by rapid degradation of this 3-phenylcoumarin constitutes a new fungal catabolic route for an isoflavan skeleton. Our present hypothesis is that **4** is further catabolized by hydrolysis of the lactone ring, decarboxylation to a stilbene and cleavage to two benzoic acids. This assumption is presently under investigation.

EXPERIMENTAL

Fungus. Fusarium oxysporum Schlecht ex Fr. f. *lycopersici* (CBS 167.30) was stored and grown as previously described [2].

Incubation experiments. Incubation of substrates (10^{-4} M) with washed mycelium, UV spectroscopic measurements of metabolite formation and isolation of catabolites were according to published methods [2–5].

Substrate. The synthesis of (±)-medicarpin has been described [20].

Chromatography. TLC on silica gel was performed with the solvents S_1: CH_2Cl_2–MeOH, 15:2, and S_2:CH_2Cl_2–MeOH, 10:2. Compounds were detected with diazotized *p*-nitroaniline.

Spectroscopy. Techniques and equipment for recording UV, ^{1}H NMR (300 MHz, TMS as int. standard) and mass spectra were as previously reported [2–4].

7,2′-*Dihydroxy*-4′-*methoxyisoflavan* (**2**). UV λ_{max}^{MeOH} nm: 280, 285; $\lambda_{max}^{MeOH+NaOMe}$ nm: 240 (sh), 293. MS *m/z* (rel. int.): 272 $[M]^+$ (10), 150 (100), 138 (22), 137 (40), 135 (15), 123 (16). ^{1}H NMR ($CDCl_3$): δ 7.01 (*d*, 1H, *J* = 8.5 Hz, H-5), 6.94 (*d*, 1H, *J* = 8.5 Hz, H-6′), 6.47 (*dd*, 1H, *J* = 8.6, 2.4 Hz, H-6), 6.35–6.4 (*m*, 3H, H-8, H-3′, H-5′), 4.34 (*dd*, 1H, *J* = 8.2, 2.7 Hz, H-2α), 4.04 (*t*, 1H, *J* = 9.9 Hz, H-2β), 3.46–3.53 (*m*, 1H, H-3α), 3.0 (*dd*, 1H, *J* = 15.7, 10.4 Hz, H-4β), 2.89 (*dd*, 1H, *J* = 15.2, 5.2 Hz, H-4α), 3.76 (*s*, 3H, 4′-OMe).

7,2′-*Dihydroxy*-4′-*methoxyisoflav*-3-*ene* (**3**). UV λ_{max}^{MeOH} nm: 219, 240 (sh), 325, 350 (sh); $\lambda_{max}^{MeOH+NaOMe}$ nm: 214, 250 (sh) 342. MS *m/z* (rel. int.): 270 $[M]^+$ (100), 269 (56), 255 (37), 161 (39), 148 (94), 147 (58), 137 (41), 135 (36), 134 (42), 133 (22), 128 (24), 127 (24), 126 (54), 120 (29), 119 (29), 115 (37), 105 (39). ^{1}H NMR (CD_3OD): δ 7.14 (*d*, 1H, *J* = 8.5 Hz, H-5), 6.88 (*d*, 1H, *J* = 8.1 Hz, H-6′), 6.53 (*s*, 1H, H-4), 6.42 (*dd*, 1H, *J* = 8.5, 2.5 Hz, H-6), 6.37 (*d*, 1H, *J* = 2.5 Hz, H-8), 6.33 (*dd*, 1H, *J* = 8.1, 2.3 Hz, H-5′), 6.25 (*d*, 1H, *J* = 2.2 Hz, H-3′), 4.95 (*d*, 2H, *J* = 1 Hz, 2H-2), 3.75 (*s*, 3H, 4′-OMe).

7,2′-*Dihydroxy*-4′-*methoxyisoflav*-3-*en*-2-*one* (**4**). λ_{max}^{MeOH} nm: 241, 284 (sh), 342. $\lambda_{max}^{MeOH+NaOMe}$ nm: 255 (sh), 294, 384. MS *m/z* (rel. int.): 284 $[M]^+$ (100), 256 (18), 241 (72), 213 (14), 185 (10), 157 (28), 128 (88). ^{1}H NMR (CD_3OD): δ 7.83 (*s*, 1H, H-4), 7.43 (*d*, 1H, *J* = 8.5 Hz, H-5), 7.20, (*d*, 1H, *J* = 8.0 Hz, H-6′), 6.77 (*dd*, 1H, *J* = 8.5, 2.2 Hz, H-6), 6.70 (*d*, 1H, *J* = 2.1 Hz, H-8), 6.48 (*dd*, 1H, *J* = 8.3, 2.5 Hz, H-5′), 6.46 (*d*, 1H, *J* = 2.5 Hz, H-3′), 3.78 (*s*, 3H, 4′-OMe).

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