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FUNGAL METABOLISM OF PRENYLATED FLAVONOIDS

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Key Word Index—*Ascochyta rabiei*; *Aspergillus flavus*; *Botrytis cinerea*; 6-prenylnaringenin; luteone; metabolism.

Abstract—When incubated in liquid culture with *Aspergillus flavus*, the prenylated flavanone 6-prenylnaringenin [(2*S*)-6-(3,3-dimethylallyl)-5,7,4′-trihydroxyflavanone] was converted into 2,3-dihydrodihydroxyprenyl-substituted naringenin, 6-prenylnaringenin hydrate, and dihydrofurano-substituted naringenin. The latter metabolite was also found as the major metabolite of 6-prenylnaringenin in *Botrytis cinerea* culture. Further experiments using a strain of *Ascochyta rabiei* pathogenic to chickpea, which can metabolize non-planar isoflavonoid pterocarpans, revealed that 6-prenylnaringenin gave only a minute amount of metabolites, whilst the prenylated isoflavone luteone [6-(3,3-dimethylallyl)-5,7,2′,4′-tetrahydroxyisoflavone] was slowly converted into the corresponding dihydrofuranoisoflavone. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In our previous papers, we reported that isoflavones with a 3,3-dimethylallyl (prenyl) substituted on ring-A at C-6 [1, 2] or C-8 [3], or on ring-B at C-2′ [4] or C-3′ [5] together with a pterocarpan [6] and a 3-methoxyflavone [4] were variously metabolized by the fungus *Aspergillus flavus* to give hydrates [from luteone (**1**) wighteone (**2**) and topazolin (**3**)] and derivatives possessing dihydrofurano, dihydropyrano or 2,3-dihydrodihydroxyprenyl side-attachments from **1**, **2**, **3**, licoisoflavone A (**4**), 2,3-dehydrokievitone (**5**), **6**, piscidone, piscerythrone and 2′-hydroxylupalbigenin (= 3′-prenyl-luteone) [7]. Almost all the above-mentioned substrates were also metabolized by *Botrytis cinerea* into similar metabolites to those of *A. flavus* apart from the hydrates. However, lupinifolinol (**7**) possessing both pyrano and prenyl side-attachments on ring-A of 3-hydroxyflavanone was recovered unchanged from cultures of *A. flavus* and *B. cinerea* [4].

The present study using the non-planar prenylated flavanones, monoprenylnaringenins (**8**–**10**) and 6,8-diprenylnaringenin (**11**), and the prenyl isoflavones, luteone (**1**) and erythbigenin (**12**), as substrates for the metabolic experiments in the cultures of *A. flavus*, *B. cinerea* and *Ascochyta rabiei* was undertaken to determine (a) if prenylflavanones other than lupinifolinol (**7**) are metabolized or not, and (b) if *A. rabiei*, which is known as a chickpea pathogen and is able to detoxify chickpea phytoalexins, medicarpin [8] and maackianin [9], metabolizes a non-planar prenylated flavanone (6-prenylnaringenin, **8**) faster than a planar prenylated isoflavone (luteone, **1**). 6-Prenylnaringenin (**8**) was first synthesized [10] and a little later isolated from hard resins of hops, *Humulus lupulus* L. as a potent growth inhibitor against *Trichophyton* spp., the causal agent of athlete's foot [11]. Although the distribution and biological function of complex flavanones have been well reviewed [12], nothing is known about their fungal metabolism.

RESULTS AND DISCUSSION

6-*Prenylnaringenin metabolites in the cultures of* Aspergillus flavus *and* Botrytis cinerea

To the 5th day culture of *A. flavus* was added 50 ppm of (2*S*)-6-prenylnaringenin, prepared from (*S*)-naringenin [13], and 3 days later the metabolic culture was terminated. The TLC patterns of flavonoids, in the ethyl acetate extract of this metabolic culture were compared with those of the control culture without the substrate. As shown in Table 1, four UV 254 nm light quenching spots, slowly yielding a purplish blue–dark blue pigment with Gibbs reagent, were observed on thin-layer plates [14]. The Gibbs test response was indicative of the presence of a *para*-unsubstituted and H-bonded phenolic group (C-5-OH) as with the substrate [15]. These metabolites were isolated by preparative TLC and chemically characterized by the

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Table 1. 6-Prenylnaringen in metabolites in some fungal cultures

Substrate and metabolite	Yields in *A. flavus* culture from 65 mg of substrate	R_f on silica gel 60 F254		UV λ_{max} nm			
		CM = 25:1	CAAm = 70:60:1	MeOH	+NaOMe	+$AlCl_3$	+NaOAc
6-Prenylnaringenin (**8**)	-	0.22	0.45	294	331	316 366	331
AF-6PN-1 (**13**) (= BC-6PN-1*)	6.7	0.09	0.22	294	326	315 369	327
AF-6PN-2 (**14**)	7.6	0.13	0.30	294	329	312 363	329
AF-6PN-3 (**15**) (= BC-6PN-2† = AR-6PN-1)	12.9	0.23	0.51	296	295‡ 417‡	319 362	296
AF-6PN-4 (**16**)	trace	0.22	0.43	—	—	—	

TLC solvent systems: CM = $CHCl_3$–MeOH; CAAm = $CHCl_3$–$(CH_3)_2CO$–concd aq. ammonia.
* 0.5 mg from 10 mg of the substrate (**8**).
† 4.2 mg from 10 mg of **8**.
‡ Probably decomposed into the corresponding chalcone.

systematic physico-chemical procedures proposed previously [7].

The following physico-chemical properties of AF-6PN-1 (**13**): UV absorption maximum shifts with NaOAc (+33 nm, 7-OH) and $AlCl_3$ (+21 nm, 5-OH) [15], molecular weight (M^+ at m/z 374 = substrate+O+H_2O) and mass fragments at m/z 356 $[M-H_2O]^+$ and 285 $[M-89]^+$, ^1H NMR protons attributable to a 2,3-dihydroxy-3-methylbutyl unit (two methyls around δ 1.2–1.3, a set of methylene protons, two double doublets around δ 2.5 and 3.1, and a methine proton on the hydroxylated carbon at δ 3.6) [7] clearly indicated that the metabolite consisted of a glycol side-chain and the naringenin skeleton depicted in **13**.

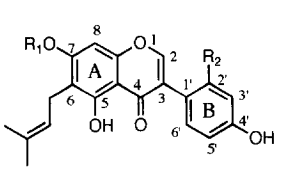
The second metabolite AF-6PN-2 (**14**) possessing unsubstituted 5-OH and 7-OH (bathochromic shifts of UV absorption maximum with $AlCl_3$ and NaOAc) [15] together with the molecular weight (M^+ at m/z 358 = substrate+H_2O) and a mass fragment at m/z 285 $[M-73]^+$ was clearly the hydration product of 6-prenylnaringenin [7]. The ^1H NMR detection of α- and β-methylenes from the aromatic ring-A at δ 2.66 and 1.66, and two singlet methyls on a hydroxylated carbon at δ 1.24 unambiguously revealed the presence of a 3-hydroxy-3-methylbutyl side-chain [7]. The second metabolite was thus confirmed to be 6-(3-hydroxy-3-methyl)naringenin (**14**).

The major metabolite AF-6PN-3 (**15**) (M^+ at m/z 356 = substrate+O) showed prominent mass fragments at m/z 59 (100%) and 297 ($[M-59]^+$, 30% characteristic of a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofurano part structure [7], and no shift of UV absorption maximum on the addition of NaOAc (substituted 7-OH) [15]. Together with these physico-chemical properties, the ^1H NMR signal at δ 4.77 (*dd*, J = 9 and 8) assignable not to H-3 in a 2,2-dimethyl-3-hydroxychromane (usually at δ *ca* 3.8) but to H-2 in a 2-alkyl-2,3-dihydrobenzofuran part structure [3, 7], clearly suggested the exact structure of AF-6PN-3 (**15**).

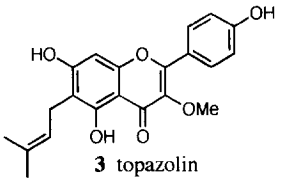
The presence of some additional ^1H NMR signals in Table 2, for example, Ha-3, H-8, Ha-1″, Hb-1″ and OH-5 in AF-6PN-1, and H-2, Ha-3 and OH-5 in AF-6PN-3, was presumably due to epimerization at an asymmetric centre in the side-attachments [13]. Considering the results of our earlier studies on the stereochemistry of prenylisoflavone metabolism [16, 17], the epimerization seems to take place in the culture or during isolation.

The final metabolite AF-6PN-4 was obtained in a trace amount and ^1H NMR spectroscopy revealed the isolate to be a mixture of the substrate and a metabolite which afforded M^+ at m/z 356 and mass fragments at m/z 340 $[M-H_2O]^+$, 323 $[M-(H_2O+CH_3)]^-$ and 285 $[M-71]^+$ characteristic of fungal metabolites possessing the 2,2-dimethyl-3-hydroxychromane part structure shown in **16** [7].

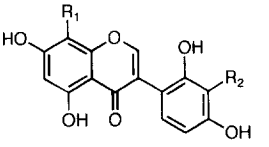
A further metabolic experiment using 6-prenylnaringenin (**8**) and *B. cinerea* culture was con-



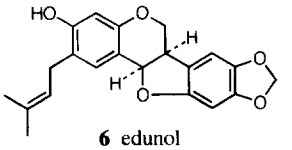
1 R_1=H, R_2=OH, luteone
2 R_1=R_2=H, wighteone
18 R_1=Me, R_2=OH, 7-*O*-methyl-luteone



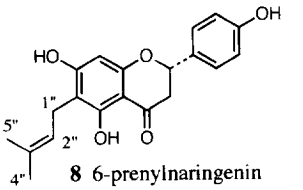
3 topazolin



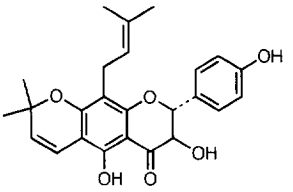
4 R_1=H, R_2=prenyl, licoisoflavone A
5 R_1=prenyl, R_2=H, 2,3-dehydrokievitone



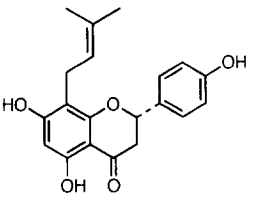
6 edunol



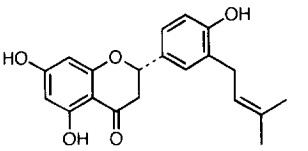
8 6-prenylnaringenin



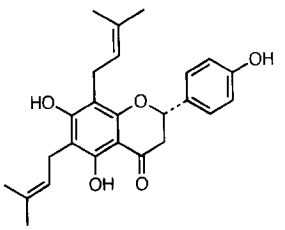
7 lupinifolinol



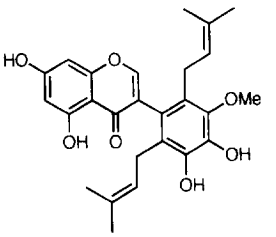
9 8-prenylnaringenin



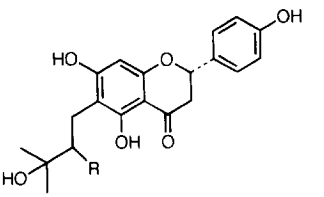
10 3'-prenylnaringenin



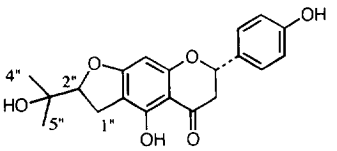
11 lonchocarpol A



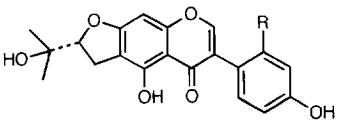
12 erythbigenin



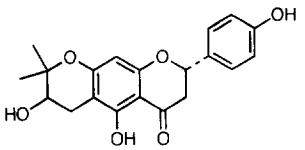
13 R=OH, AF-6PN-1 (=BC-6PN-1)
14 R=H, AF-6PN-2



15 AF-6PN-3 (=BC-6PN-2)



17 R=OH, AR-L-1 (=lupinisoflavone B)
19 R=H, erythrinin C



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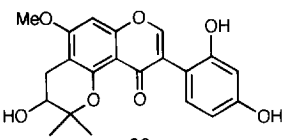

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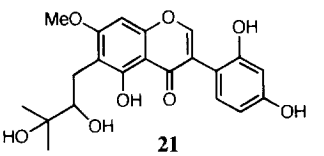

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ducted similarly and revealed that the substrate (10 mg) was converted into BC-6PN-1 (=AF-6PN-1, **13**) and BC-6PN-2 (=AF-6PN-3, **15**) in 0.5 and 4.2 mg yields, respectively.

Metabolism of prenylated flavonoids in Ascochyta rabiei *cultures*

Ascochyta rabiei, a pathogenic fungus to chickpea, shows a high potentiality to detoxify simple isoflavones [18] and pterocarpans [8, 9], through hydroxylation at bridgehead carbons and other aromatic carbons, and through *O*-demethylation, reductive cleavage of the dihydrofurano ring in the pterocarpan skeleton and decomposition of the isoflavone ring system. However, nothing has been reported about the metabolism of prenylated flavonoids by the fungus.

In the present study, the metabolism of prenylated flavanones and isoflavones in the culture of *A. rabiei* was examined by comparing it with that in *A. flavus* and *B. cinerea* cultures. In the culture of *A. rabiei* (pH 6.0), luteone [6-(3,3-dimethylallyl)-5,7,2′,4′-tetrahydroxyisoflavone, **1**] was converted slowly to a metabolite exhibiting the same R_f as that of lupinisoflavone B (**17**) [19] (a major metabolite of luteone in the *B. cinerea* culture [1]). Five and a half days after the addition of the substrate, the metabolic culture afforded the metabolite in 13% yield together with

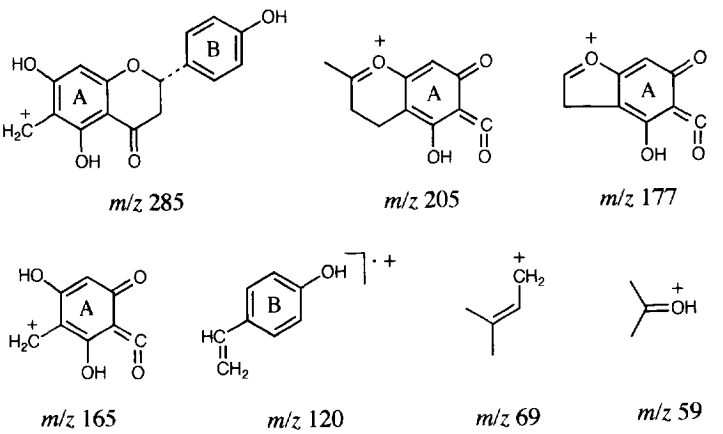

Fig. 1. Major mass fragments described in the text.

Table 2. ^{1}H NMR data for 9-prenylnaringenin (6-PN) and its fungal metabolites

Compound Proton	^{1}H NMR δ_{TMS} ppm (acetone-d_6 500 MHz)			
	6-PN (**8**)	AF-6PN-1 (**13**)	AF-6PN-2 (**14**)	AF-6PN-3 (**15**)
H-2	5.45 (1H) *dd J* = 13, 3.0	5.44 (1H) *dd J* = 13, 3.0	5.42 (1H) *dd J* = 13, 3.0	5.45/5.46 *dd* (1H in total) *J* = 13, 3.0
Ha-3	2.70 (1H) *dd J* = 17, 3.0	2.74/2.75 *dd* (1H in total) *J* = 17, 3.0	2.72 (1H) *dd J* = 17, 3.0	2.72/2.74 *dd* (1H in total) *J* = 17, 3.0
Hb-3	3.17 (1H) *dd J* = 17, 13	3.18 (1H) *dd J* = 17, 13	3.17 (1H) *dd J* = 17, 13	3.18 (1H) *dd J* = 17, 13
H-8	6.03 (1H) *s*	5.97/5.98 *s* (1H in total)	6.00 (1H) *s*	5.91 (1H) *s*
H-2′ & H-6′	7.39 (2H) *br.d J* = 8.6	7.39 (2H) *d*-like *J* = 8.5	7.40 (2H) *d J* = 8.5	7.40 (2H) *d J* = 8.5
H-3′ & H-5′	6.90 (2H) *br.d J* = 8.6	6.89 (2H) *d*-like *J* = 8.5	6.90 (2H) *J* = 8.5	6.90 (2H) *d J* = 8.5
Ha-1″	3.25 (2H) *br.d J* = 7.3	2.48/2.50 *dd* (1H in total) *J* = 14, 9.7	2.66 (2H) *m*	3.03 (1H) *dd J* = 16, 9.6
Hb-1″		3.12/3.14 *dd* (1H in total) *J* = 14, 2.0		3.08 (1H) *dd J* = 16, 7.8
Hn-2″ (*n* = 1 or 2)	5.24 (1H) *br.t J* = 7.3	3.58 (1H) *dd*-like *J* = 9.7, 2.0	1.66 (2H) *m*	4.77 (1H) *dd* *J* = 9.6, 7.8
H_3-4″	1.64 (3H) *s*	1.24 (3H) *s*	1.24 (6H) *s*	1.22 (3H) *s*
H_3-5″	1.75 (3H) *s*	1.26 (3H) *s*		1.26 (3H) *s*
5-OH	12.47 (1H) *s*	12.64/12.67 *s* (1H in total)	12.48 (1H) *s*	12.33/12.35 *s* (1H in total)

Duplicated signals of Ha-3, H-8, Ha-1″, Hb-1″ and OH-5 in AF-6PN-1, and H-2, Ha-3 and OH-5 in AF-6PN-3 seem to be due to the presence of diastereoisomers. One of disatereoisomers of AF-6PN-3 isolated by HPLC afforded signals for H-2, Ha-3 and OH-5 at δ 5.46, 2.74 and 12.33, respectively.

major amounts of unchanged substrate (**1**), and at that point no other metabolites were found on thin-layer chromatograms. The mass spectrum of the isolate showed M^+ at *m/z* 370 ($C_{20}H_{18}O_7$ = substrate + O) and mass fragments at *m/z* 311 ($[M-59]^+$, 81%) and 59 (100%) characteristic of 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofurano side-attachment ([1, 7] and see the MS of AF-6PN-3). The UV maximum of the metabolite at 263 nm was shifted with $AlCl_3$ (+8 nm, free 5-OH) and unchanged with NaOAc (substituted 7-OH) [15] as with AF-6PN-3 (**15**). The metabolite was thus concluded to be a dihydrofurano derivative of luteone (**1**), and finally confirmed to be identical with lupinisoflavone B including its stereochemistry [16] by direct comparison of the TLC, UV, MS and ORD data with those of the authentic compound [15, 19].

When incubated in the *A. rabiei* culture for 5 days, a minute amount of 6-prenylnaringenin (**8**) was converted into a metabolite apparently identical to AF-6PN-3 in behaviour (R_f and Gibbs test response) on thin-layer chromatograms.

Substrate acceptability of prenylated flavonoids to the fungal metabolism

Preliminary metabolic experiments revealed that *A. rabiei*, in general, metabolized some prenylated isoflavones more slowly than *A. flavus* or *B. cinerea* did. For example, when added into the *B. cinerea* or *A. flavus* culture (pre-cultured for 4 days), 50 ppm of luteone (**1**) was completely metabolized in 2 or 3 days [1] into some products all possessing a modified side-attachment. However, in the culture of *A. rabiei* (pre-cultured for 6–7 days), only minute amounts of a metabolite were detected on thin-layer chromatograms three days after the addition of the substrate into the culture and the yield of the metabolite after 5.5 days was 13%. Other prenylated isoflavones, wighteone (**2**), licoisoflavone A (**4**), and 7-*O*-methyl-luteone (**18**) were also metabolized by the fungus as slowly as luteone (**1**). The latter substrate was converted into two metabolites indistinguishable from the corresponding dihydropyran (**20**) and glycol (**21**) on thin-layer plates [20]. However, no luteone (**1**) or luteone metabolites anticipated to be produced via *O*-demethylation were detected in the metabolites.

The non-planar substrates, 6-prenylnaringenin (**8**) and 3′-prenylnaringenin (**10**) were also metabolized in *A. rabiei* cultures far more slowly than luteone (**1**) was. In these cases, only small amounts of metabolite(s) were observed in the culture five days after the addition of each substrate. 8-Prenylnaringenin (**9**) and 6,8-diprenylnaringenin (= lonchocarpol A, **11**) in the *A. rabiei* culture, and erythbigenin (**12**) in *B. cinerea* and *A. flavus* cultures yielded practically no metabolites in the present study. Although both *A. flavus* and *B. cinerea* afforded no metabolites from lupinifolinol, a 3-hydroxyflavanone possessing both prenyl and dimethylpyrano side-attachments (**7**), the present study apparently revealed that these fungi could metabolize a monoprenylflavanone, 6-prenylnaringenin (**8**) into the corresponding hydrate (**14**) and dihydrofurano (**15**) and glycol (**13**) derivatives similar to those from prenylated isoflavones.

It is not surprising that *A. rabiei*, compatible with chickpea yielding pterocarpan phytoalexins [21, 22] as defense substances, possesses a poor detoxifying activity against antifungal prenylated flavonoids common in lupins [23]. The key step in the metabolism of these prenylated flavonoids in fungi is recognized as an epoxidation at the prenyl double bond [17, 24], and the reaction has recently been demonstrated in a cell-free system from *B. cinerea* pretreated with 6-prenylnaringenin (**8**) [25].

EXPERIMENTAL

General procedures (e.g. silica gel TLC and UV) were undertaken using the equipment and conditions previously described [1, 4]. Mass and ^{1}H NMR spectra were recorded on a JEOL JMS-DX300 using a direct inlet system and on a Bruker AM500 at 500 MHz in $(CD_3)_2CO$, respectively.

Substrate and other chemicals. Monoprenylated (2*S*)-naringenins substituted at C-6 (**8**), C-8 (**9**) and C-3′ (**10**) were prepared from commercially available (2*S*)-naringenin [13]. Lupin isoflavones [wighteone (**2**), luteone (**1**) and licoisoflavone A (**4**)], lonchocarpol A (**11**), and erythbigenin (**12**) were isolated from the white lupin roots [19], yellow lupin roots [13], and the root bark of *Piscidia erythrina* [26], respectively. 7-*O*-Methyl-luteone was derived from **1** by methylation with diazomethane [27], and its fungal metabolites have been already reported [20].

Fungi and metabolic experiments. Aspergillus flavus AHU 7049, *Botrytis cinerea* AHU 9424 and *Ascochyta rabiei* strain 3 were cultured for 4–7 days in a shaking liquid medium consisting of glucose (5 g), peptone (1 g), yeast extract (0.1 g) and tap water (100 ml). A solution of each substrate (5 or 6 mg in 1 ml of EtOH) was then added, and after a further incubation for 3–5 days the metabolites and any remaining substrate were extracted from the medium with EtOAc [see refs 1, 5].

Isolation and purification of 6-prenylnaringenin metabolites. An EtOAc extract of the combined *Aspergillus* culture medium [1300 ml, initially containing 65 mg of 6-prenylnaringenin (**8**)] or *Botrytis* culture medium (200 ml, 10 mg of **8**) was washed with 5% aqueous $NaHCO_3$, and then with a saturated solution of NaCl. After removal of the EtOAc *in vacuo*, the residue was chromatographed (silica gel analytical and preparative TLC) in $CHCl_3$–MeOH = 25:1 and $CHCl_3$–$(Me)_2CO$–concd aq. ammonia (70:60:1) to afford 4 metabolites from *Aspergillus* culture and two from *Botrytis* culture, and the remaining substrates all detectable as UV 254 nm quenching and Gibbs test positive (purple-blue) spots on silica gel 60 F_{254} thin-layer plates. Yields and chromatographic and UV spectral properties are summarized in Table 1.

Metabolites in Ascochyta rabiei *cultures.* An EtOAc extract of *Ascochyta rabiei* strain 3 culture medium [200 ml, initially containing 12 mg of luteone (**1**)] was worked-up similarly to that of the *A. flavus* culture, and the major metabolite (AR-L-1) indistinguishable from lupinisoflavone B [19] (= luteone metabolite BC-1 [1]) on thin-layer plates and UV and ORD spectra was isolated in 13% yield. In a further metabolic experiment using prenylated flavonoids in *Ascochyta* cultures, the formation of dihydrofurano metabolites from wighteone and 6-prenylnaringenin (**19** [2] and **15**), and 7-*O*-methyl-luteone metabolites (**20** and **21**) from 7-*O*-methyl-luteone was only demonstrated on thin-layer plates by direct comparison with authentic compounds. Small amounts of Gibbs positive metabolites were also detected on thin-layer chromatograms of EtOAc extracts from *Ascochyta* cultures administered with licoisoflavone A (**4**) and 3′-prenylnaringenin (**10**).

Isolated metabolites. UV, ^{1}H NMR and chromatographic properties of fungal metabolites and 6-

prenylnaringenin are shown in Tables 1 and 2. Major mass fragments are depicted in the text [28]. *AF-6PN-1* (**13** = *BC-6PN-1*): Gibbs test, (+), slow, purple blue. MS *m/z* (rel. int.): 374 $[M]^+$ (0.5), 356 (10), 285 $[M-89]^+$ (33), 195 (19), 166 (20), 165 (100), 150 (13), 123 (16), 120 (45), 119 (14), 107 (14), 91 (22), 69 (27), 65 (19), 59 (40). *AF-6PN-2* (**14**): Gibbs test, (+), slow, dark blue. MS *m/z* (rel. int.): 358 $[M]^+$ (4), 340 $[M-H_2O]^+$ (18), 285 (29), 220 (15), 205 (18), 192 (14), 179 (13), 166 (12), 165 (100), 123 (17), 120 (36), 91 (12), 69 (20). *AF-6PN-3* (**15** = *BC-6PN-2*): Gibbs test, (+), slow, purple blue. MS *m/z* (rel. int.): 356 $[M]^+$ (44), 323 (32), 298 (33), 297 $[M-59]^+$ (30), 203 (60), 179 (30), 178 (72), 177 (65), 176 (33), 165 (36), 150 (87), 120 (57), 91 (24), 69 (30), 59 (100). *AR-L-1* (**17**): Gibbs test, (+), rapid, blue–purple. $[\alpha]_D^{23}-93^\circ$ (*c* 0.11; MeOH), MS *m/z* (rel. int.): 370 $[M]^+$ (86), 337 (36), 312 (50), 311 $[M-59]^+$ (81), 179 (28), 177 (27), 150 (21), 134 (29), 59 (100).

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REFERENCES

1. Tahara, S., Nakahara, S., Mizutani, J. and Ingham, J. L., *Agricultural and Biological Chemistry*, 1984, **48**, 1471.
2. Tahara, S., Nakahara, S., Ingham, J. L. and Mizutani, J., *Nippon Nogeikagaku Kaishi*, 1985, **59**, 1039.
3. Tahara, S., Misumi, E., Mizutani, J. and Ingham, J. L., *Zeitschrift für Naturforschung*, 1987, **42c**, 1055.
4. Tahara, S., Ingham, J. L. and Mizutani, J., *Zeitschrift für Naturforschung*, 1991, **46c**, 341.
5. Tahara, S., Nakahara, S., Mizutani, J. and Ingham, J. L., *Agricultural and Biological Chemistry*, 1985, **49**, 2605.
6. Tahara, S. and Ingham, J. L., *Zeitschrift für Naturforschung*, 1987, **42c**, 1050.
7. Nakahara, S., Tahara, S., Mizutani, J. and Ingham, J. L., *Agricultural and Biological Chemistry*, 1986, **50**, 863.
8. Kraft, B., Schwenen, L., Stöckl, D. and Barz, W., *Archives of Microbiology*, 1987, **147**, 201.
9. Hohl, B., Arnemann, M., Schwenen, L., Stöckl, D., Bringmann, G., Jansen, J. and Barz, W., *Zeitschrift für Naturforschung*, 1989, **44c**, 771.
10. Nagar, A., Gujral, V. K. and Gupta, S. R., *Tetrahedron Letters*, 1978, 2031.
11. Sekiguchi, S. and Sato, Y., *Agricultural and Biological Chemistry*, 1984, **48**, 2771.
12. Bohm, B. A., in *The Flavonoids: Advances in Research Since 1986*, ed. J. B. Harborne. Chapman and Hall, London, 1994, pp. 387–440.
13. Tahara, S., Katagiri, Y., Ingham, J. L. and Mizutani, J., *Phytochemistry*, 1994, **36**, 1261.
14. King, F. E., King, T. J. and Manning, L. C., *Journal of the Chemical Society*, 1957, 563.
15. Mabry, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavonoids*. Springer, Berlin, 1970, p. 169.
16. Tahara, S., Ingham, J. L. and Mizutani, J., *Agricultural and Biological Chemistry*, 1987, **51**, 211.
17. Tahara, S., Saitoh, F. and Mizutani, J., *Zeitschrift für Naturforschung*, 1993, **48c**, 16.
18. Kraft, B. and Barz W., *Applied and Environmental Microbiology*, 1985, **50**, 45.
19. Tahara, S., Ingham, J. L., Nakahara, S., Mizutani, J. and Harborne, J. B., *Phytochemistry*, 1984, **23**, 1889.
20. Tahara, S., Ingham, J. L. and Mizutani, J., *Nippon Nogeikagaku Kaishi*, 1989, **63**, 999.
21. Barz, W. and Welle, R., *Recent Advances in Phytochemistry*, 1992, **26**, 139.
22. Mackenbrock, U. and Barz, W., *Zeitschrift für Naturforschung*, 1991, **46c**, 43.
23. Tahara, S., Shibaki, S., Ingham, J. L. and Mizutani, J., *Zeitschrift für Naturforschung*, 1990, **45c**, 147.
24. Tahara, S., Ingham, J. L. and Mizutani, J., *Phytochemistry*, 1989, **28**, 2079.
25. Tanaka, M., Mizutani, J. and Tahara, S., *Bioscience, Biotechnology and Biochemistry*, 1996, **60**, 171.
26. Tahara, S., Moriyama, M., Ingham, J. L. and Mizutani, J., *Phytochemistry*, 1991, **30**, 2769.
27. Tahara, S., Watanabe, K. and Mizutani, J., *Journal of the Faculty of Agriculture Hokkaido University*, 1987, **63**, 155.
28. Mabry, T. J. and Markham, K. L., in *The Flavonoids*, ed. T. J. Mabry, J. B. Harborne and H. Mabry. Chapman and Hall, London, 1975, p. 78.