Substrate Specificity in the Fungal Metabolism of Prenylated Flavonoids

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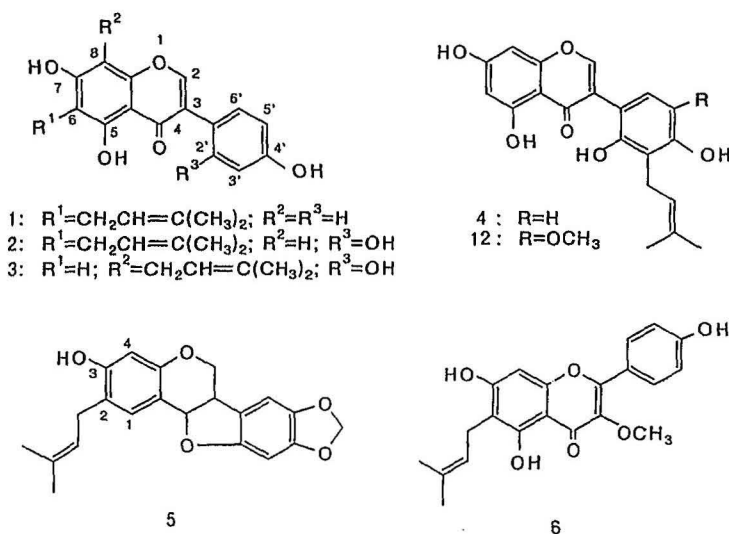P.O. Box 226, Reading RG 6 2 AP, England

The prenylated flavonoids, topazolin [5,7,4'-trihydroxy-3-methoxy-6-(3,3-dimethylallyl)-flavone], piscerythrone [5,7,2',4'-tetrahydroxy-5'-methoxy-3'-(3,3-dimethylallyl)isoflavone] and piscidone [5,7,4',5'-tetrahydroxy-2'-methoxy-6'-(3,3-dimethylallyl)isoflavone] were metabolized by *Aspergillus flavus* and *Botrytis cinerea* to give a variety of products. Topazolin and piscerythrone were converted by both fungi to compounds similar to those previously obtained from luteone (6-prenyl) and licoisoflavone A (3'-prenyl) respectively. The 6'-prenylated isoflavone piscidone was metabolized only by *B. cinerea* to give the corresponding dihydropyrano-isoflavone as a major product. Neither fungus was found to metabolize the 8-prenylated pyranoflavanonol lupinifolinol.

Introduction

In earlier papers we reported that 6-, 8- and 3'-prenylated isoflavones (wighteone (1) [1] and luteone (2) [2], 2,3-dehydrokievitone (3) [3], and licoisoflavone A (4) [4]) and the 2-prenylated pterocarpan edunol (5) [5] were variously metabolized by *Aspergillus flavus* and *Botrytis cinerea* to give less fungitoxic hydrates, glycols and cyclic ether derivatives. These metabolites can be assumed to arise by one of two basic reactions, namely prenyl hydration by *A. flavus* (→ hydrates) or prenyl oxidation by both fungi (→ cyclic ethers and glycols). The latter reaction presumably involves the initial formation of an unstable intermediate epoxide [6] from which a glycol sidechain or a cyclic ether (dihydrofurano or dihydropyrano) derivative can easily be obtained.

Our isolation of a 6-prenylated flavone (topazolin (6)) from *Lupinus luteus* cv. Topaz [7], an 8-prenylated pyranoflavanonol (= 3-hydroxyfla-

1: R¹=CH₂CH=C(CH₃)₂; R²=R³=H
2: R¹=CH₂CH=C(CH₃)₂; R²=H; R³=OH
3: R¹=H; R²=CH₂CH=C(CH₃)₂; R³=OH

4 : R=H
12 : R=OCH₃

5

6

7 (AT-1)

8 (BT-1)

9 (BT-2)

10 (BT-3)

11

13 R=OCH$_3$ (PEM-1)
18 R=H (M-1-2)

14 R=OCH$_3$ (PEM-2)
19 R=H (M-2)

15 R=OCH$_3$ (PEM-3)
20 R=H (M-1-1)

16 R=OCH$_3$ (PEM-4)
21 R=H (M-3-1)

17 R=OCH$_3$ (piscerythrone glycol)
22 R=H (M-3-2)

23

24 (BP-1)

vanone) (lupinifolinol (**11**)) from *Lonchocarpus guatemalensis* [8], and the prenylated isoflavones piscerythrone (**12**) (3′-prenyl) and piscidone (**23**) (6′-prenyl) from *Piscidia erythrina* [9, 10] prompted us to conduct further metabolic studies using these new substrate molecules.

The present paper describes the isolation and identification of compounds resulting from the fungal metabolism of topazolin (**6**), piscerythrone (**12**) and piscidone (**23**). The fourth substrate, lupinifolinol (**11**), was recovered unchanged from cultures of both *A. flavus* and *B. cinerea*.

Results and Discussion

Although topazolin (**6**) is a flavone, it possesses an A-ring part structure (5,7-dihydroxy-6-prenyl)

identical with that of the isoflavones wighteone (**1**) and luteone (**2**) previously used in our metabolic studies [1, 2]. When incubated with *B. cinerea*, topazolin (10 mg) was converted into three metabolites denoted BT-1 (**8**, 4.5 mg), BT-2 (**9**, 0.7 mg) and BT-3 (**10**, 2.3 mg). In the presence of *A. flavus*, the same quantity of topazolin principally yielded metabolite AT-1 (**7**, 7.9 mg) together with trace amounts of the *Botrytis* metabolites BT-1 and BT-3.

^{1}H NMR studies indicated that differences between these metabolites and the substrate topazolin were restricted to the A-ring side structure (Table I). From MS data, metabolites AT-1 (M^+ 386 = substrate + 18 mass units), BT-1 and BT-2 (both M^+ 384 = substrate + 16 mass units), and BT-3 (M^+ 402 = substrate + 34 mass units) were provi-

Table I. ^{1}H NMR data for topazolin (**6**), piscidone (**23**) and their metabolites[a].

Proton	Topazolin (**6**)	AT-1 (**7**)	BT-1 (**8**)	BT-2 (**9**)	BT-3 (**10**)	Piscidone (**23**)	BP-1 (**24**)
2-H	–	–	–	–	–	7.91 s	8.05 s
3-OMe	3.86 s (3H)	3.87 s (3H)	3.87 s (3H)	3.87 s (3H)	3.87 s (3H)	–	–
5-O<u>H</u>	13.08 s	13.08 s	13.01 s	13.13 s	13.26 s	13.01 s	12.98
6-H	–	–	–	–	–	6.29 d $J = 2.2$	6.31 d $J = 1.9$
8-H	6.56 s	6.54 s	6.46 s	6.44 s	6.53 d[b] $J = 1.7$	6.43 d $J = 2.2$	6.45 d $J = 1.9$
2′-H	8.01 br. d $J = 9.0$ (2H)	8.02 br. d $J = 9.0$ (2H)	8.04 d $J = 8.9$ (2H)	8.04 d $J = 9.0$ (2H)	8.05 d $J = 8.9$ (2H)	–	–
6′-H						–	–
3′-H	7.01 br. d $J = 9.0$ (2H)	7.02 br. d $J = 9.0$ (2H)	7.02 d $J = 8.9$ (2H)	7.02 d $J = 9.0$ (2H)	7.02 d $J = 8.9$ (2H)	6.41 s	6.53 s
5′-H						–	–
2′-OMe	–	–	–	–	–	3.79 s (3H)	3.80 s (3H)
1″-Ha	3.36 br. d	2.78 m	3.14 dd $J = 15.6, 9.4$	2.63 dd $J = 16.9, 7.2$	2.60 dd $J = 14.5, 9.9$	3.25 br. d	2.59 dd $J = 16.0, 8.4$
1″-Hb	$J = 7.3$ (2H)	(2H)	3.19 dd $J = 15.6, 7.7$	2.95 dd $J = 16.9, 5.3$	3.24 br. d $J = 14.5$	$J = 6.6$ (2H)	2.79 dd $J = 16.0, 5.7$
2″-H	5.28 br. t $J = 7.3$	1.71 t-like m (2H)	4.84 dd $J = 9.4, 7.7$	3.87 incomplete[c]	3.65 dd $J = 9.9, 1.9$	5.07 br. t $J = 6.6$	3.73 dd $J = 8.4, 5.7$
4″-Me	1.79 s (3H)	1.26 s (6H)	1.25 s (3H)	1.33 s (3H)	1.26 s (3H)	1.43 br. s (3H)	1.26 s (3H)
5″-Me	1.65 d $J = 1.0$ (3H)		1.29 s (3H)	1.39 s (3H)	1.29 s (3H)	1.51 d $J = 1.2$ (3H)	1.39 s (3H)

[a] All spectra were determined in acetone-d_6 at 100 MHz (**6** and **23**) or at 500 MHz (**7**–**10** and **24**). Coupling constants (*J*) are in Hz. For multiplets, the δ value indicates the centre of the signal.
[b] The reason for the observed weak coupling of 8-H is unclear.
[c] The signal overlapped with 3-OMe.

sionally identified as topazolin hydrate (**7**), cyclic ether derivatives of topazolin (**8** and **9**), and topazolin glycol (**10**) respectively.

Metabolite AT-1 was indistinguishable (UV, MS, ¹H NMR and TLC) from authentic topazolin hydrate (**7**) previously isolated from the roots of *L. luteus* [7]. The presence of a glycol (2,3-dihydroxy-3-methylbutyl) sidechain in metabolite BT-3 (**10**) was evident from the MS fragment at m/z 313 (M⁺ − 89), and this was confirmed by a ¹H NMR comparison with the known glycol derivatives of wighteone [1], luteone [2] and 2'-hydroxylupalbigenin [11]. Differentiation between the cyclic ether side structures of metabolites BT-1 (**8**) and BT-2 (**9**) was based on the chemical shift value of the methine (C-2″) proton which resonated at δ 4.84 in BT-1 [2-(1-hydroxy-1-methylethyl)-2,3-dihydrofurano side structure] and at δ 3.87 in BT-2 (2,3-dihydro-3-hydroxy-2,2-dimethylpyrano side structure) [2]. Further evidence for each side structure was provided by a ¹H NMR comparison with luteone metabolites BC-1 and BC-2 respectively [2], and by the characteristic MS fragments at m/z 325 (M⁺ − 59; BT-1) [1, 2] and m/z 313 (M⁺ − 71; BT-2) [1, 2].

In contrast to topazolin, neither *A. flavus* nor *B. cinerea* metabolized the 8-prenylated pyranoflavanonol lupinifolinol (**11**, 10 mg) which was recovered unchanged from the culture media. The reason for the failure to metabolize lupinifolinol is unclear, although the non-planarity of the basic flavanone ring system seems unlikely to be a major factor. Thus, whilst isoflavones have a planar ring system, we have shown that the non-planar pterocarpan edunol (**5**; prenyl at C-2) is metabolized by *A. flavus* to give dihydrofurano-, dihydropyrano- and glycol-derivatives [5].

Our earlier studies on the root bark components of *P. erythrina* [9] yielded large quantities of the isoflavone piscerythrone (**12**) (= 5'-methoxylicoisoflavone A). Although the metabolism of licoisoflavone A by *A. flavus* and *B. cinerea* has been reported elsewhere [4], it was of interest to determine if either fungus could demethylate an isoflavone such as **12**, in addition to modifying the prenyl attachment.

When incubated with *A. flavus* or *B. cinerea*, piscerythrone ((**12**), 10 mg) was converted into four compounds PEM-1−4 (**13−16**) identifiable from MS and ¹H NMR data as the 5'-methoxy deriva-

tives of licoisoflavone A metabolites M-1-2 (**18**), M-2 (**19**) (both dihydrofurano isoflavones cyclized 3'→4'[O] and 3'→2'[O] respectively), M-1-1 (**20**) and M-3-1 (**21**) (both dihydropyrano isoflavones cyclized as in M-1-2 and M-2) [4]. The four piscerythrone metabolites (see experimental) gave ¹H NMR signals similar to those reported for the corresponding licoisoflavone A derivatives (**18−21**) [4] except for the presence in each spectrum of an additional 3 H signal at δ 3.75−3.80 s (OCH₃) and the absence of one of two aromatic protons for B-ring. Molecular weights (M⁺) of the piscerythrone metabolites were increased over those of the licoisoflavone A compounds by the expected 30 mass units. As with the licoisoflavone A study [4], cultures of *A. flavus* yielded predominantly the 5'-methoxy dihydrofurano derivatives (**13, 14**) of piscerythrone, whilst *B. cinerea* afforded the 5'-methoxy dihydropyrano derivatives (**15, 16**) as major metabolites. Cultures of *A. flavus* and *B. cinerea* also contained traces of a fifth metabolite which could not be identified. However, its position on thin-layer chromatograms relative to that of piscerythrone and the four identifiable metabolites suggested that it might possibly be piscerythrone glycol (**17**) (= 5'-methoxylicoisoflavone A glycol). Both *A. flavus* and *B. cinerea* have previously been found to convert licoisoflavone A (**4**) into a glycol derivative (**22**) [4].

No evidence was obtained to show that piscerythrone could be demethylated by the isolate of *B. cinerea* used in the present study. In contrast, previous work involving a different isolate of *B. cinerea* suggests that this fungus can demethylate the isoflavonoid (pterocarpan) medicarpin to give demethylmedicarpin [12].

In addition to piscerythrone, we have also investigated the fungal metabolism of piscidone (**23**), a 6'-prenylated isoflavone similarly obtained from *P. erythrina* [9, 10]. Piscidone (**23**) and kwakhurin from *Pueraria mirifica* [13] appear to be the only monoprenylated isoflavones with the prenyl group at C-6' (= 2'). Although the fungal modification of isoflavones monoprenylated at C-6 [1, 2], C-8 [3] and C-3' (= 5') [12, and ref. 4] has already been reported, no work has yet involved compounds with 6'-prenylation.

In the presence of *B. cinerea* (but not *A. flavus*) the sterically hindered 6'-prenyl group of piscidone (25 mg) was slowly modified to yield metabolite

BP-1 (9.4 mg) and two very minor products (BP-2 and BP-3). A substantial quantity of piscidone (approx. 11 mg) was recovered unchanged from the medium.

Metabolite BP-1 (M^+ 400 = substrate + 16 mass units) was identified as the 2,3-dihydro-3-hydroxy-2,2-dimethylpyrano isoflavone (24) from the characteristic ^1H NMR value (δ 3.73) of the methine proton (C-2'') [2]. MS fragments at m/z 367 ($M^+ - 33$), m/z 341 ($M^+ - 59$) and m/z 329 ($M^+ - 71$) also supported structure 24 [2]. Apart from side structure differences, the ^1H NMR spectrum of BP-1 closely resembled that of piscidone (Table I). We believe that metabolites BP-2 and BP-3 are probably the dihydrofurano derivative corresponding to 24, and piscidone glycol respec-

tively, but neither compound could be obtained in amounts sufficient for identification.

The substrate specificity in fungal metabolism of prenylated flavonoids/isoflavonoids is summarized in Fig. 1. Hydration of the prenyl sidechain was only associated with metabolism by *A. flavus*, and was confined to the sidechain at C-6 (ring A). In other respects, *A. flavus* and *B. cinerea* were generally similar, giving cyclic ether and glycol metabolites in varying amounts regardless of whether the substrate was prenylated at C-6/C-8 on ring A, or at C-3' (= 5') on ring B. However, as mentioned earlier, only *B. cinerea* appeared capable of modifying a prenyl group located at C-6' (ring B). The 6-prenyl of the flavone topazolin (6) and the 2-prenyl sidechain (corresponding to C-6 in isofla-

isoflavone   flavone   pterocarpan

A. prenylation at C-6

   wighteone (1)
   luteone (2)
   topazolin (6, flavone)
   2'-hydroxylupalbigenin [11]

B. prenylation at C-6, C-8,
   C-3' (=5') and C-6' (=2')

   wighteone (1)
   luteone (2)
   2,3-dehydrokievitone (3)
   licoisoflavone A (4)
   topazolin (6, flavone)
   piscerythrone (12)
   piscidone (23)
   2'-hydroxylupalbigenin [11]

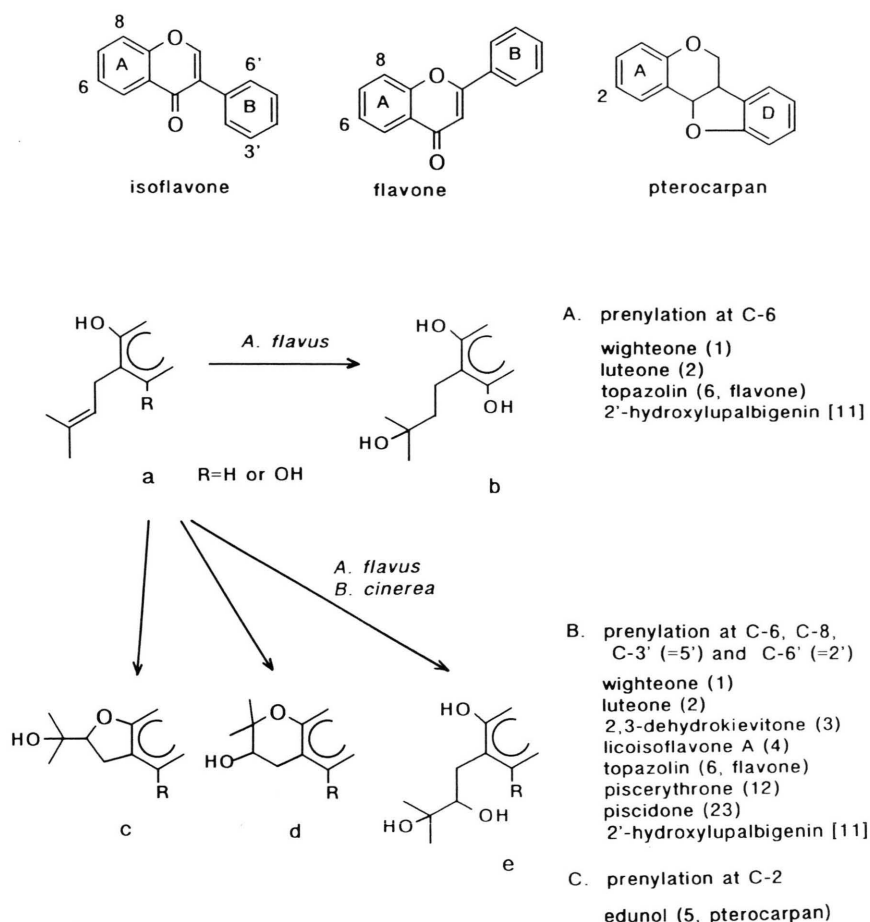C. prenylation at C-2

   edunol (5, pterocarpan)

Fig. 1. Substrate specificity in the fungal metabolism of prenylated flavonoids.

vones/flavones) of the pterocarpan edunol (**5**), were also found to be susceptible to modification by *A. flavus* or *B. cinerea* [this paper and ref. 5].

Experimental

For general details of the metabolic experiments, and extraction of compounds etc. see our earlier papers [1–5]. Substrates were added to 4-day old liquid cultures of *A. flavus* AHU 7049 and *B. cinerea* AHU 9424 [1] at a level of 5 mg compound/100 ml medium. After a further 3 days incubation, the metabolites were extracted [1] and separated by silica gel PTLC. *Rf* values relative to that of the substrate (r*Rf*s) were determined by multiple development (× 3–4) TLC using Merck silica gel 60 plates (F-254, 0.25 mm thickness) in $CHCl_3$–MeOH (CM).

Metabolism of topazolin (**6**; *10 mg/culture*)

An EtOAc extract of topazolin metabolites from *A. flavus* cultures was separated by silica gel PTLC (CM, 50:3) to afford AT-1 (**7**; 7.9 mg, major product) and traces of BT-1 and BT-2. A mixture of *Botrytis* metabolites was separated in a similar manner to give BT-1 (**8**; 4.5 mg), BT-2 (**9**; 0.7 mg) and BT-3 (**10**; 2.3 mg). r*Rf*s in CM (20:1) were: AT-1 0.48; BT-1, 1.22; BT-2, 1.13; and BT-3, 0.38.

Metabolism of lupinifolinol (**11**; *10 mg/culture*)

Lupinifolinol (**11**) was recovered unchanged from the culture medium after 4 days incubation with *A. flavus* or *B. cinerea*.

Metabolism of piscerythrone (**12**; *10 mg/culture*)

Cultures of *A. flavus* and *B. cinerea* were each found to contain five metabolites. Four of these were isolated (silica gel PTLC in CM 50:3) and identified as two dihydrofurano isoflavones (major products from *A. flavus* cultures; PEM-1, 5.6 mg and PEM-2, 2.4 mg) and two dihydropyrano isoflavones (major products from *B. cinerea* cultures; PEM-3, 2.1 mg and PEM-4, 2.5 mg). The fifth metabolite, which could not be isolated, was provisionally identified as a glycol derivative. The dihydrofurano and dihydropyrano metabolites corresponded respectively with the 5'-methoxy derivatives of licoisoflavone A metabolites M-1-2 (**18**)/

M-2 (**19**) and M-1-1 (**20**)/M-3-1 (**21**) [4]. r*Rf*s in CM (20:1) were as follows: PEM-1, 0.57; PEM-2, 0.54; PEM-3, 0.50; and PEM-4, 0.43, and the fifth metabolite (piscerythrone glycol?), 0.16.

Metabolism of piscidone (**23**; *25 mg/culture*)

Silica gel PTLC (CM, 50:3) of *B. cinerea* culture extracts afforded metabolite BP-1 (**24**; 9.4 mg) and traces of two other compounds (BP-2 and BP-3). Large quantities of unmetabolized substrate (11.2 mg) were also recovered. Piscidone was not metabolized by *A. flavus*. r*Rf*s in CM (20:1) were: BP-1, 0.62; BP-2, 0.50 and BP-3, 0.20.

Physicochemical properties of topazolin (**6**), *piscerythrone* (**12**), *and piscidone* (**23**) *metabolites*

^{1}H NMR data for compounds **6** and **23**, and their metabolites, are shown in Table I.

AT-1 (**7**): Yellow plates, m.p. 247–248 °C (lit. [7], 239–241 °C). UV_{365nm} fluorescence: dull black. Gibbs test: green-blue → purple. MS m/z (rel. int.): 386 (M^+; 13), 369 (5.8), 368 (M^+ – 18; 25), 367 (7.7), 353 (6.1), 351 (5.2), 328 (5.2), 327 (14), 326 (7.8), 325 (M^+ – 61; 38), 314 (21), 313 (M^+ – 73; 100); 312 (17), 311 (13), 310 (5.5), 298 (6.3), 270 (7.3), 269 (7.2), 123 (6.7), 121 (16), 69 (5.9). UV λ_{max}, nm: MeOH 214.5, 272, 285–310 (br.), 338 (br.); +NaOMe 235sh, 276.5, 314sh, 330, 399; +$AlCl_3$ 212, 235sh, 278, 307, 358, 405sh; +NaOAc 274, 301, 314sh, 372 (H_3BO_3 regenerated the MeOH spectrum).

BT-1 (**8**): Pale yellow needles, m.p. 181–183 °C. UV_{365nm} fluorescence: dull black. Gibbs test: green-blue → blue. MS m/z (rel. int.): 385 (M^+ + 1; 23), 384 (M^+; 91), 383 (42), 366 (7.9), 365 (7.6), 352 (7.6), 351 (35), 326 (33), 325 (M^+ – 59; 100), 311 (11), 310 (11), 308 (10), 307 (23), 297 (13), 295 (7.6), 283 (9.1), 269 (7.3), 131 (13), 121 (32), 93 (7.2), 59 (49). UV λ_{max}, nm: MeOH 217, 261sh, 269, 308sh (br.), 343 (br.); +NaOMe 236sh, 262sh, 270.5, 300sh (br.), 391 (br.); +$AlCl_3$ 217, 229sh, 268sh, 277.5, 303sh, 309, 367, 392sh; +NaOAc unchanged.

BT-2 (**9**): Pale yellow fine needles, m.p. 200–203 °C. UV_{365nm} fluorescence: dull black. Gibbs test: blue. MS m/z (rel. int.): 385 (M^+ + 1; 23), 384 (M^+; 100), 383 (45), 365 (13), 351 (9.7), 314 (15), 313 (M^+ – 71; 76), 312 (41), 311 (8.3), 298 (7.9), 297 (13), 295 (12), 283 (8.6), 270 (11), 269 (31), 131

(10), 121 (32), 93 (8.0), 71 (7.2), 69 (11), 65 (7.2), 57 (21), 55 (14). UV λ_{max}, nm: MeOH 213, 270, 308 sh (br.), 335−355 (br.); +NaOMe 206.5, 233 sh, 263 sh, 271, 300 sh, 398; +AlCl$_3$ 213, 271, 300 sh (br.), 335−355 (br.); +NaOAc 264 sh, 271, 305.5 (br.), 372 (br.), 390 sh (partly decomposed ?).

BT-3 (**10**): Pale yellow fine needles, m.p. 219−221 °C. UV$_{365 nm}$ fluorescence: dull black. Gibbs test: blue. MS m/z (rel. int.): 402 (M$^+$; 3.5), 384 (11), 344 (16), 343 (M$^+$ − 59; 63), 314 (29), 313 (M$^+$ − 89; 100), 312 (5.4), 300 (7.9), 299 (7.8), 298 (6.0), 270 (6.7), 123 (8.3), 121 (13), 97 (12), 69 (5.9), 59 (6.0), 55 (9.5): UV λ_{max}, nm: MeOH 214, 271, 290−305 (br.), 342 (br.); +NaOMe 204, 233 sh, 276, 319 sh, 328, 399; +AlCl$_3$ 212, 232 sh, 281, 294−300 (br.), 307, 362, 394 sh; +NaOAc 276, 301.5, 308 sh, 377.5 (br.) (H$_3$ BO$_3$ regenerated the MeOH spectrum).

PEM-1 (**13**): Pale yellow plates, m.p. 284−286 °C. UV$_{365 nm}$ fluorescence: dark purple. Gibbs test: gray-green → blue. MS m/z (rel. int.): 401 (M$^+$ + 1; 26), 400 (M$^+$; 100), 382 (19), 367 (49), 342 (26), 341 (M$^+$ − 59; 64), 329 (28), 328 (36), 327 (21), 313 (15), 203 (15), 153 (29), 59 (29). UV λ_{max}, nm: MeOH 207, 261, 295 (br.); +NaOMe 204, 270, 320 (br.); +AlCl$_3$ 207, 270, 290 sh (br.), 310 sh (br.), 373 (br.); +NaOAc 272, 295 sh, 325 sh (br.) (H$_3$ BO$_3$ regenerated the MeOH spectrum). ^{1}H NMR δ_{TMS} (acetone-d_6, 500 MHz, J = Hz): 1.25 and 1.30 (both 3H, two s, 4″- and 5″-H$_3$), 3.15 (1H, dd, J = 15.7, 9.6, 1″-Ha); 3.23 (1H, dd, J = 15.7, 8.4, 1″-Hb), 3.80 (3H, s, 5′-OMe), 4.72 (1H, br. t, J = ca. 9, 2″-H), 6.34 (1H, d, J = 2.2, 6-H), 6.48 (1H, d, J = 2.2, 8-H), 6.75 (1H, s, 6′-H), 8.24 (1H, s, 2-H), 12.66 (s, 5-O$\underline{H}$).

PEM-2 (**14**): Colourless fine needles, m.p. 279−281 °C. UV$_{365 nm}$ fluorescence: dark purple. Gibbs test: brown → dark purple-blue. MS m/z (rel. int.): 400 (M$^+$; 15), 383 (12), 382 (50), 368 (17), 367 (76), 343 (17), 342 (86), 341 (M$^+$ − 59; 100), 313 (12), 309 (20), 203 (12), 153 (32), 91 (19), 79 (13), 77 (13), 69 (15), 59 (17). UV λ_{max}, nm: MeOH 206, 262, 300 (br.); +NaOMe 205, 272, 307 (br.), 320 sh (br.); +AlCl$_3$ 205, 225 sh, 271, 302 (br.), 371 (br.); +NaOAc 270, 305 (br.), 320 sh (H$_3$ BO$_3$ regenerated the MeOH spectrum). ^{1}H NMR δ_{TMS} (acetone-d_6, 500 MHz, J = Hz): 1.20 and 1.29 (both 3H, two s, 4″- and 5″-H$_3$); 3.14 (1H, dd, J = 15.8, 9.4, 1″-Ha), 3.22 (1H, dd, J = 15.8, 7.5, 1″-Hb); 3.79 (3H, s, 5′-OMe), 4.64 (1H, dd, J = 9.4, 7.5, 2″-H),

6.29 (1H, d, J = 2.0, 6-H), 6.43 (1H, d, J = 2.0, 8-H), 7.09 (1H, s, 6′-H), 8.40 (1H, s, 2-H), 13.05 (s, 5-O$\underline{H}$).

PEM-3 (**15**): Pale yellow plates, m.p. 251−254 °C. UV$_{365 nm}$ fluorescence: dark purple. Gibbs test: gray-green → blue. MS m/z (rel. int.): 401 (M$^+$ + 1; 26), 400 (M$^+$; 100), 382 (18), 367 (45), 330 (28), 329 (M$^+$ − 71; 69), 328 (75), 315 (16), 313 (35), 285 (20), 203 (18), 153 (32), 69 (15). UV λ_{max}, nm: MeOH 205, 262, 295 (br.); +NaOMe 202, 274, 300 sh, 330 sh (br.); +AlCl$_3$ 268, 290 sh, 310 sh (br.), 367 (br.); +NaOAc 274, 295 sh (br.), 330 sh (br.) (H$_3$ BO$_3$ regenerated the MeOH spectrum). ^{1}H NMR δ_{TMS} (acetone-d_6, 500 MHz, J = Hz): 1.28 and 1.39 (both 3H, two s, 4″- and 5″-H$_3$), 2.63 (1H, dd, J = 17.2, 7.9, 1″-Ha), 3.01 (1H, dd, J = 17.2, 5.6, 1″-Hb), 3.75 (3H, s, 5′-OMe), 3.80 (1H, m, 2″-H), 6.35 (1H, d, J = 2.2, 6-H), 6.51 (1H, d, J = 2.2, 8-H), 6.74 (1H, s, 6′-H), 8.30 (1H, s, 2-H, 12.56 (s, 5-O$\underline{H}$).

PEM-4 (**16**): Colourless glassy solid. UV$_{365 nm}$ fluorescence: dark purple. Gibbs test: gray-green → dark purple-blue. MS m/z (rel. int.): 401 (M$^+$ + 1; 30), 400 (M$^+$; 100), 382 (16), 367 (43), 341 (19), 330 (37), 329 (M$^+$ − 71; 80), 328 (69), 315 (19), 285 (16), 203 (13), 153 (29), 69 (13). UV λ_{max}, nm: MeOH 206, 260, 296 (br.); +NaOMe 205, 268, 305 (br.), 320 sh (br.); +AlCl$_3$ 203, 225 sh, 268, 300 (br.), 367 (br.); +NaOAc 267, 298, 320 sh (br.) (H$_3$BO$_3$ regenerated the MeOH spectrum). ^{1}H NMR δ_{TMS} (acetone-d_6, 500 MHz, J = Hz): 1.22 and 1.29 (both 3H, two s, 4″- and 5″-H$_3$), 2.61 (1H, dd, J = 17.2, 7.9, 1″-Ha), 3.01 (1H, dd, J = 17.2, 5.8, 1″-Hb), 3.76 (1H, br. t, J = ca. 7, 2″-H), 3.80 (3H, s, 5′-OMe), 6.28 (1H, d, J = ca. 2, 6-H), 6.42 (1H, d, J = ca. 2, 8-H), 6.90 (1H, s, 6′-H), 8.07 (1H, s, 2-H), 13.16 (s, 5-O$\underline{H}$).

BP-1 (**24**): Pale yellow glassy solid. UV$_{365 nm}$ fluorescence: dark. Gibbs test: dark blue. MS m/z (rel. int.): 400 (M$^+$; 8.2), 383 (7.5), 382 (27), 368 (27), 367 (100), 342 (17), 341 (15), 330 (11), 329 (M$^+$ − 71; 19), 327 (9.5), 301 (17), 153 (66), 69 (6.5). UV λ_{max}, nm: MeOH 208, 255 sh, 261, 295, 325 sh (br.); +NaOMe 205, 270, 329 (br.); +AlCl$_3$ 209, 226 sh, 269, 311, 365; +NaOAc 270, 329 (br.) (H$_3$ BO$_3$ regenerated the MeOH spectrum).

the MS spectra. The fungal isolates used in the present study were kindly supplied by Prof. S. Takao, Dept. of Agric. Chem., Hokkaido Univ.

Note added in proof:

Further investigation has revealed the structure **23** for piscidone incorrect. Consequently, the structure of piscidone metabolite BP-1 (**24**) must be revised according to the exact structure of piscidone [5,7,3′,4′-tetrahydroxy-5′-methoxy-2′-(3,3-dimethylallyl)isoflavone].

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