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IDENTIFICATION OF AN EPOXY-INTERMEDIATE RESULTING FROM THE FUNGAL METABOLISM OF A PRENYLATED ISOFLAVONE

SATOSHI TAHARA, JOHN L. INGHAM* and JUNYA MIZUTANI

Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060, Japan; *Department of Food Science, University of Reading, Whiteknights, P.O. Box 226, Reading RG6 2AP, U.K.

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Key Word Index—*Botrytis cinerea*; Hyphomycetes; 7-*O*-methyl-2,3-dehydrokievitone epoxide; 7-*O*-methyl-2,3-dehydrokievitone glycol; prenylated isoflavone; fungal metabolism; stereochemistry.

Abstract—The prenylated isoflavone 2,3-dehydrokievitone was methylated with ethereal diazomethane to yield the corresponding 7-*O*-methyl derivative. This was metabolized by *Botrytis cinerea* to give 7-*O*-methyl-2,3-dehydrokievitone epoxide and 7-*O*-methyl-2,3-dehydrokievitone glycol. The structures of these metabolites were established by standard spectroscopic methods (UV, MS and ^{1}HNMR), and by chemical conversion. Both compounds were found to possess the *S*-absolute stereochemistry. The epoxide is an analogue of the key metabolic intermediate thought to be involved in the formation, by fungi, of various dihydrofurano-, dihydropyrano- and 2,3-dihydroxy-3-methylbutyl-substituted isoflavones from precursor compounds containing a prenyl side-chain with *ortho*-hydroxylation.

INTRODUCTION

In a previous paper [1], we reported that the 8-prenylated isoflavone 2,3-dehydrokievitone (**1**) was metabolized by two fungi, *Aspergillus flavus* and *Botrytis cinerea*, to yield compounds **5** (dihydrofurano-isoflavone), **6** (dihydropyrano-isoflavone) and **7** (2,3-dihydrodihydroxyprenyl-isoflavone), all of which might arise via epoxidation of the unsaturated side-chain in the substrate (Fig. 1). The 6- and/or 3′-prenylated isoflavones wighteone [2], luteone [3], 2′-hydroxylupalbigenin [4] and licoisoflavone A [5] were also converted by both fungi into compounds comparable with those derived from **1**.

A variety of complex isoflavones with pyrano, dihydrofurano and dihydropyrano side-attachments are known to occur in *Lupinus* (Leguminosae) [6–8], and these substituents also appear in other groups of natural products including terpenoids [10], coumarins [11] and alkaloids [12]. In order to explain the biogenesis of these side-attachments, epoxidation of the prenyl substituent by a mono-oxygenase is usually proposed [13]. However, despite precise experiments on the biosynthesis of complex isoflavonoids such as rotenone [14] and the glyceollins [15, 16], no epoxide intermediates have so far been identified.

In our paper dealing with the fungal metabolism of luteone [3], we proposed that the prenylated substrate and its cyclic ether and glycol (2,3-dihydrodihydroxyprenyl) derivatives were linked by a transitory epoxide intermediate. We further suggested that if this was the case, luteone metabolism could provide a useful model for the mechanistic investigation of E-ring formation during the biosynthesis of rotenone. However, it has already been reported [17] that epoxidation of a prenyl side-chain with *ortho* hydroxylation results in a highly active molecule from which cyclic ethers are rapidly formed by interaction of the epoxide ring with the adjacent OH substituent. Hydrolysis to give a glycol may also occur in aqueous solution, and both this process and cyclic ether formation are enhanced by acid conditions (Scheme 1). Thus, if intermediate epoxides are formed during the fungal metabolism of isoflavones such as luteone and wighteone, it is perhaps not surprising that as yet they have proved impossible to isolate from liquid cultures. Amongst the isoflavonoids, psoralidin oxide [3,9-dihydroxy-2-(2′,3′-epoxy-3′-methylbutyl)coumestan] from seeds of *Psoralea corylifolia* [18] is, to our knowledge, the only known compound with an epoxide side-chain and an *ortho* OH group.

As exemplified by our study on luteone [3], all the A-ring cyclic ether metabolites of 5,7-dihydroxy-6-prenylisoflavones have a side structure arrangement (C-6→C-7[O]) which reflects the low reactivity of the H-bonded C-5 OH group. Attempts to detect an epoxide intermediate using 7-*O*-methyl-luteone were unsuccessful, metabolism by *B. cinerea* leading only to the detection of 7-*O*-methyl-luteone glycol, and cyclic ethers with a C-6→C-5[O] ring system [19]. However, as described here, an epoxide intermediate was eventually isolated from cultures of *B. cinerea* following brief incubation with the substrate isoflavone 7-*O*-methyl-2,3-dehydrokievitone [5,2′,4′-trihydroxy-7-methoxy-8-(3,3-dimethylallyl)isoflavone, **2**].

RESULTS AND DISCUSSION

2,3-Dehydrokievitone [5,7,2′,4′-tetrahydroxy-8-(3,3-dimethylallyl)isoflavone, **1**] was first obtained as a minor phytoalexin from the *Monilinia fructicola*-inoculated pods of *Phaseolus vulgaris* [20]. The same compound has also been isolated from methanol extracts of yellow lupin

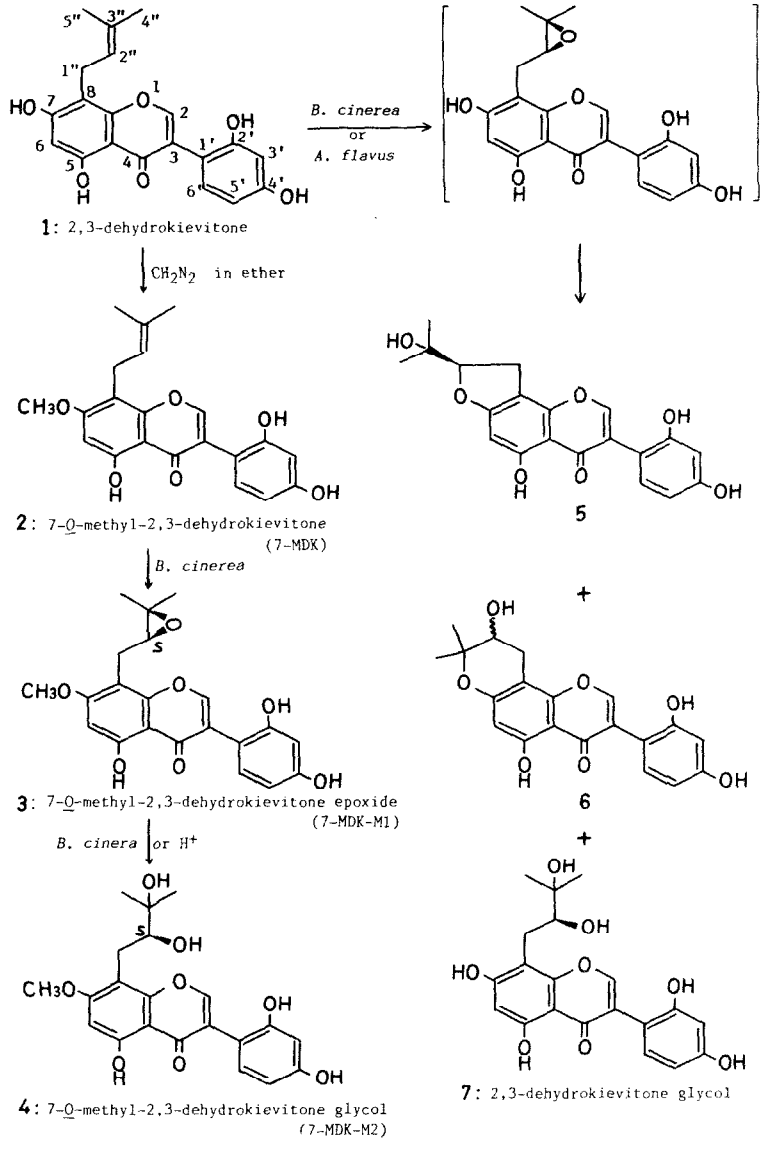


Fig. 1. Comparative metabolism of 2,3-dehydrokievitone (**1**) and 7-*O*-methyl-2,3-dehydrokievitone (**2**) by *Botrytis cinerea*. The epoxide in brackets is a possible intermediate with *S*-configuration. Metabolite **6** was isolated as an optically inactive form [1].

(*Lupinus luteus*) roots [21]. In the present study, 2,3-dehydrokievitone (170 mg) of *Lupinus* origin was dissolved in methanol–ether, and then methylated with ethereal diazomethane to preferentially yield the desired 7-*O*-methyl ether (= 7-MDK, **2**, 110 mg). This compound, and other minor methyl ethers of **1**, were isolated by TLC as described in the Experimental.

We reported earlier [1] that **1** was metabolized more slowly than its regio-isomers luteone [3] and licoisoflavone A [5] when incubated for four days in a shaking liquid medium containing freshly cultured *Aspergillus flavus* or *Botrytis cinerea*. Interestingly, however, our present results show that **2** is metabolized *ca* 10 times faster than **1** by *B. cinerea*, although no obvious decrease in substrate level was evident after four days incubation with *A. flavus*.

The isoflavonoids variously isolated after incubation of **2** with *B. cinerea* (see Experimental for details) are shown diagrammatically in Fig. 2. No Gibbs reagent-positive compounds were isolated from *Botrytis* cultures grown for eight days in the absence of **2** (Fig. 2, lane 1). When incubated with *B. cinerea* for 24 hr or longer, its rapid disappearance (reference material, lane 7) was coupled with the detection of a major Gibbs reagent-positive substance (denoted 7-MDK-M2) at very low R_f (lanes 4, 5 and 6). However, sampling of the culture medium at 6 and 12 hr (lanes 2 and 3 respectively) revealed, in addition to 7-MDK-M2, a second Gibbs positive spot immedi-

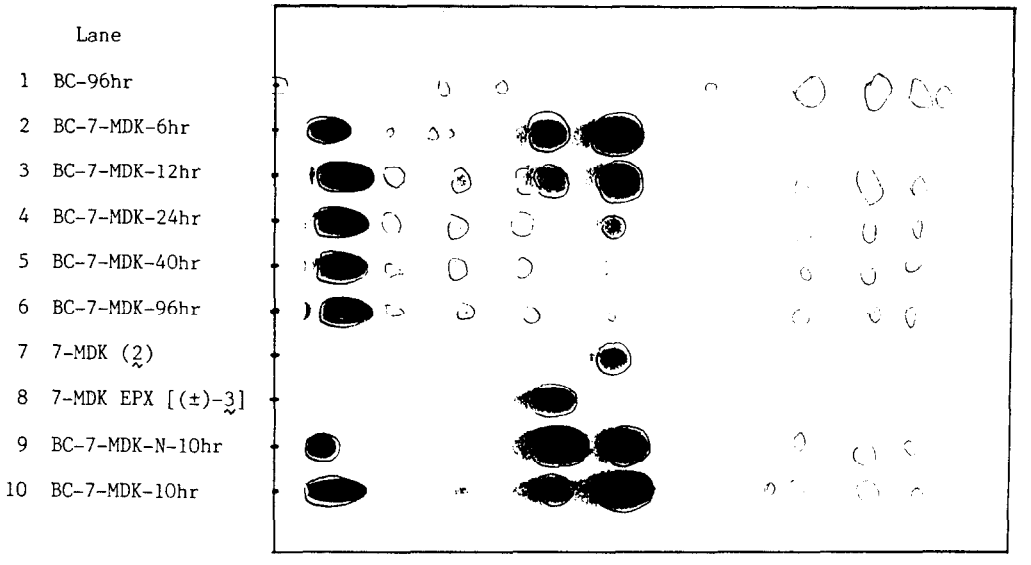
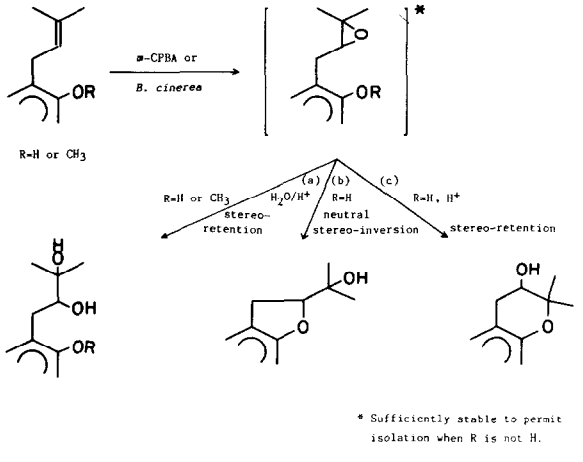


Fig. 2. Thin-layer detection of compounds arising from the metabolism of 7-*O*-methyl-2,3-dehydrokievitone (**2**) by *Botrytis cinerea*. Solvent; CAAm (70 : 60 : 1). Abbreviations: BC = *Botrytis cinerea* precultured for 4 days (pH 4.1). 7-MDK = 7-*O*-methyl-2,3-dehydrokievitone (substrate). EPX = epoxide derivative of 7-MDK. N = pH of media adjusted to near neutral (6.7) before addition of 7-MDK.

Suffixed numbers indicate the length of time (hr) that cultures were incubated following addition of 7-MDK (substrate), except for lane 1 (control) where cultures were shaken for 96 hr in the absence of 7-MDK. Authentic compounds are located in lanes 7 (7-MDK, **2**) and 8 (7-MDK epoxide, **3**). Compounds were detected by fluorescence quenching when thin-layer chromatograms were examined under UV (245 nm) light (circled areas), and by the coloured zones formed after treatment with Gibbs reagent [7, 30] (shaded areas).

ately below the unchanged 7-MDK. This second metabolite (7-MDK-M1) was provisionally identified as 7-*O*-methyl-2,3-dehydrokievitone epoxide (**3**) from a TLC comparison with chemically prepared material running in lane 8.

The low running material was considered to be the glycol (**4**) on the basis of our previous work which has shown that prenylated isoflavones are typically metabolized by *B. cinerea* to yield either a glycol (Scheme 1, a), or a cyclic ether derivative (Scheme 1, b and c). However,



Scheme 1. Chemical and biological transformation of *ortho*-prenyl-phenolic compounds.

since **2** does not possess an OH group *ortho* to the prenyl side-chain, the identity of this metabolite as a cyclic ether can be effectively discounted leaving a glycol as the most likely structure.

During the four days that *B. cinerea* was growing in liquid culture prior to the addition of substrate (see Experimental), the pH of the medium changed from an initial value of 6.4 to a more acidic 4.1. Under these acid conditions, it is possible that an epoxy intermediate may undergo hydrolysis resulting in a decreased yield. To test this possibility, a study of metabolite formation under essentially neutral conditions was carried out. The pH of the *B. cinerea* pre-culture broth was therefore adjusted to 6.7 immediately before addition of the substrate isoflavone. No change in the pH level of the medium occurred over the subsequent 10 hr incubation period. As expected, TLC of the resulting metabolites (Fig. 2) revealed that in a neutral medium the ratio of the epoxy compound to the glycol was somewhat greater (lane 9) than in a culture of unmodified (acidic) pH (lane 10). The two metabolites **3** and **4** were isolated and purified by TLC prior to spectroscopic (UV, MS, ^{1}H NMR) investigation. Both compounds were found to be slightly laevorotatory.

The upper metabolite was firmly identified as 7-*O*-methyl-2,3-dehydrokievitone epoxide (**3**) by direct comparison (UV, MS, TLC) with authentic material prepared chemically from compound **2**. ^{1}H NMR signals were assigned as shown in Table 1, and were also entirely consistent with the proposed epoxide structure.

The mass spectrum of the more polar metabolite afforded a molecular ion at *m/z* 402 (substrate + 2 × OH),

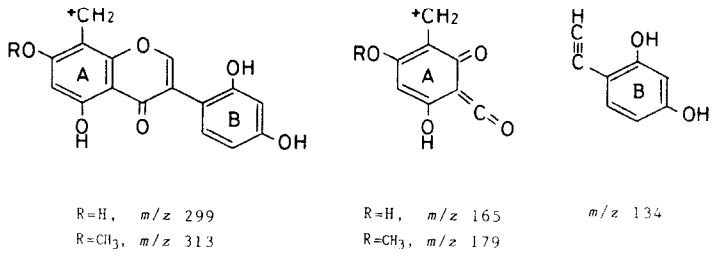
Table 1. ^{1}H NMR data (δ values) for 7-*O*-methyl-2,3-dehydrokievitone, its metabolites, and the related compound 2,3-dehydrokievitone*

H	2.3-Dehydro-kievitone **1** (100 MHz)	7-*O*-Methyl-2,3-dehydrokievitone (**2**)	7-*O*-Methyl-2,3-dehydrokievitone epoxide (**3**)	7-*O*-Methyl-2,3-dehydrokievitone glycol (**4**)
2-H	8.27 *s*	8.27 *s*	8.28 *s*	8.26 *s*
5-OH	12.70 *s*	12.83 *s*	12.92 *s*	12.88 *s*
6-H	6.40 *s*	6.53 *s*	6.57 *s*	6.54 *s*
7-OMe	—	3.98 *s* (3H)	4.01 *s* (3H)	3.98 *s* (3H)
3′-H	6.49 *d* (i)†	6.49 *d* *J* = 2.4	6.48 *d* *J* = 2.4	6.49 *d* *J* = 2.4
5′-H	6.44 *dd* (i)	6.45 *dd* *J* = 8.3, 2.4	6.45 *dd* *J* = 8.3, 2.4	6.45 *dd* *J* = 8.3, 2.4
6′-H	7.15 *br d* *J* = 8.8	7.14 *d* *J* = 8.3	7.15 *d* *J* = 8.3	7.15 *d* *J* = 8.3
1″-Ha			3.08 *dd*-like *J* = 16.6, 7.5	2.971 *d*-like *J* = 8.3
1″-Hb	3.47 *br d* (2H) *J* = 7.3	3.45 *br d* (2H) *J* = 7.2	2.88 *dd*-like *J* = 16.6, 7.3	2.967 *d*-like *J* = 4.5
2″-H	5.26 *br t* *J* = 7.3	5.19 *br t*-like *J* = 7.2	2.88 *t*-line *J* = *ca* 7.3	3.61–3.67 *ddd*-like *J* = 8.3, 5.7, 4.5
4″-H$_3$	1.81 *s* (3H)	1.80 *s* (3H)	1.41 *s* (3H)	1.25 *s* (3H)
5″-H$_3$	1.66 *s* (3H)	1.65 *d* (3H) *J* = 0.8	1.21 *s* (3H)	1.26 *s* (3H)
Phenolic OH		8.39 *s* 8.30 *s*	8.40 *s* 8.29 *s*	
Alcoholic OH				3.39 *d* *J* = 5.7 (2″-OH) 3.34 *s* (3″-OH)

*Except where indicated the spectra were determined at 500 MHz (acetone-d_6; TMS reference). *J* are in Hz.
†An incomplete signal for which a coupling constant could not be calculated.

and a base peak at *m/z* 313 $[M-89]^+$ typical of the 2,3-dihydrodihydroxyprenyl residue [3, 5]. Other major fragments were observed at *m/z* 179 (40%; RDA ion of hydroxy-methoxylated A-ring with CH_2 alkyl remnant) and *m/z* 134 (11%; main B-ring RDA ion). Similar MS fragments have been associated with wighteone and luteone glycols [2, 3], licoisoflavone A metabolite M-3-2 [5] and 2,3-dehydrokievitone metabolite DK-M3 [1], all of which contain a glycol residue derived by fungal modification of a prenyl side-chain in the substrate molecule.

The precise structure of this metabolite was confirmed by ^{1}H NMR spectroscopy (Table 1). Thus, differences in chemical shifts and coupling patterns between the substrate (**2**) and the glycol were only associated with the side-chain protons. Moreover, signals attributable to the side-chain of the glycol [δ2.971, *d*-like, *J* = 8.3 Hz, and 2.967, *d*-like, *J* = 4.5 Hz (1″-CH_2); 3.64, *ddd*-like, *J* = 8.3, 5.7, 4.5 Hz (with D_2O, *dd*, *J* = 8.2, 4.9 Hz) (2″ > CHOH); 1.26 *s*, 3H, and 1.25 *s*, 3H (2 × Me on a carbinol carbon)] closely resembled those given by 2,3-dehydrokievitone glycol **7** [1] [δ2.76 *dd*, *J* = 14.4, 10.0 Hz, and 3.29 *dd*, *J*



R=H, *m/z* 299
R=CH_3, *m/z* 313

R=H, *m/z* 165
R=CH_3, *m/z* 179

m/z 134

= 14.4, 2.0 Hz (1″-CH_2); 3.69 *dd*, *J* = 10.0, 2.0 Hz (2″ >CHOH); 1.30 *s*, 3H, and 1.29 *s*, 3H (2 × Me on a carbinol carbon)]. The small difference in chemical shift values between the methylene protons of **4** and **7** is presumably due to a larger anisotropic effect of the C-7 OH group (**7**) when compared with a methoxyl at the same position. Thus, this metabolite can be assigned structure **4**.

The close chemical relationship between metabolites **3** and **4** was also shown by the successful acid-catalysed hydrolysis of **3** to yield laevorotatory **4**. The latter compound, isolated from *Botrytis* cultures, was found to have the *S*-absolute stereochemistry at the C-2″ chiral centre by CD spectroscopy on the corresponding osmate ester [1, 20]. As the acid-catalysed hydrolysis of an epoxide ring in a 2,3-epoxy-3-methylbutyl side-chain involves stereo-retention at the chiral centre C-2 [23], it follows that **3** must also possess the *S*-configuration at C-2″. The reaction pathway, including stereochemistry, for the fungal metabolism of **2** can thus be represented as shown in Fig. 1.

Our present results support previous studies which suggest that the microbially catalysed oxidation of unsaturated carbon–carbon double bonds results in the initial formation of an epoxide which can then undergo hydrolysis to give a 1,2-glycol [24, 25]. May and Abbott [26] have also isolated a Pseudomonad enzyme system capable of converting alkenes to the corresponding epoxide in the presence of NADH and molecular oxygen. Comparable oxidation systems are also known to occur in higher plants [27]. As described in our earlier papers [7, 8, 28], white lupin roots contain a variety of dihydrofurano- and dihydropyrano-sioflavones, and it seems reasonable to suggest that these originate from the corresponding prenylated isoflavone via stereo-specific epoxidation [29].

EXPERIMENTAL

General. General procedures (e.g. UV, MS and [α] measurements, silica gel TLC and the Gibbs test) were carried out using the equipment and conditions described in our earlier papers [7, 26]. ^{1}H NMR spectra were recorded in Me_2CO-d_6 with TMS as the int. standard. Mp: uncorr. Silica gel TLC was performed using the following solvent systems: CAAm = $CHCl_3$–Me_2CO conc. aq. NH_3 (70:60:1), CM = $CHCl_3$–MeOH (10:1), BE = C_6H_6–EtOAc (3:1 or 1:1), HCE = *n*-hexane–$CHCl_3$–EtOAc (2:1:1) and CEAM = $CHCl_3$–EtOAc–Me_2CO–MeOH (15:5:5:1).

Preparation of the substrate 7-O-methyl-2,3-dehydrokievitone (**2**). 2,3-Dehydrokievitone (**1**) used in the present study was isolated from the roots of yellow lupin (*Lupinus luteus* L. cv. Topaz) as previously reported [21]. An excess of CH_2N_2 in Et_2O was added to a solution of **1** (170 mg in 10 ml MeOH–Et_2O, 1:1) under ice-cold conditions. The reaction mixture was then allowed to stand for 1 hr at 5–10° before being taken to dryness under red. pres. The resulting solid was chromatographed (PTLC) in HCE to yield 7-*O*-methyl-2,3-dehydrokievitone (**2**, R_f 0.25, 110 mg) as the major product which was eluted with EtOAc. Other minor methyl ethers (*ca* 15 mg in total) at R_f 0.73, 0.66 and 0.33, and the starting material (**1**, R_f 0.13, 25 mg) were also eluted with EtOAc. **2**: Pale yellow plates from EtOAc, mp 157–159°. $UV_{(365\,nm)}$ fluorescence, dark purple. Gibbs test: (+), rapid, purple-blue. MS *m/z* (rel. int.): 369 $[M+1]^+$ (23), 368 $[M]^+$ (84), 353 (27), 326 (22), 325 $[M-43]^+$ (100), 314 (15), 313 $[M-55]^+$ (69), 312 (14), 300 (16), 233 (16), 219 (23), 191 (14), 179 [RDA fragment of hydroxymethoxylated A-ring with CH_2 alkyl remnant] (30), 167 (13), 134 [RDA fragment of dihydroxylated B-ring] (14), 69 (13). UV λ_{max}^{MeOH} nm (ε): 218sh (36 900), 264 (40 000), 330sh (*br*) (8750); + NaOMe, 225sh, 263, 273sh, 318sh; + $AlCl_3$, 236sh, 275, 314sh, 390; + NaOAc, unchanged. ^{1}H NMR data for **2** are shown in Table 1.

Preparation of 7-O-methyl-2,3-dehydrokievitone-(±)-epoxide [(±)-**3**]. *m*-Chloroperoxybenzoic acid (10 mg) in $CHCl_3$ (0.5 ml) was added to a solution of **2** (10 mg) in $CHCl_3$ (0.5 ml) under ice-cold conditions. After stirring for 90 min at room temperature, the reaction mixture was diluted with EtOAc and then washed successively with aq. 5% $NaHCO_3$ and brine. The product was purified by PTLC in BE (1:1, R_f 0.64) to yield 6.4 mg of the required epoxide [(±)-**3**]: Pale yellow needles from EtOAc, mp 176–178°. $UV_{(365\,nm)}$ fluorescence, dark purple. Gibbs test: (+), rapid, purple-blue. MS *m/z* (rel. int.): 389 $[M+1]^+$ (9), 384 $[M]^+$ (40), 341 (27), 325 (17), 314 (20), 313 $[M-71]^+$ (100), 312 (66), 311 (11), 295 (14), 179 [RDA fragment of A-ring with CH_2 alkyl remnant] (74), 164 (13), 149 (16), 134 [RDA fragment of dihydroxylated B-ring] (15), 69 (12), 43 (13). UV λ_{max}^{MeOH} nm: 217sh, 263, 330sh (*br*); + NaOMe, 245sh, 255sh, 262, 271sh, 318sh; + $AlCl_3$, 235sh, 273.5, 313sh, 384 (*br*); + NaOAc, unchanged.

Metabolism of **2** *by* Botrytis cinerea. Cultures of *B. cinerea* AHU 9424 were grown for 4 days in a liquid medium consisting of glucose (5 g), peptone (1 g), yeast extract (0.1 g) and H_2O (100 ml) [3]. A solution of **2** (5 mg in 1 ml EtOH) was then added, and after further incubation for 6, 10, 12, 24, 40 or 96 hr (see text and Fig. 1) the metabolites were extracted from the medium with EtOAc [3]. In other experiments, the pH of the culture broth was adjusted to near neutral (6.7) before addition of **2** and incubation for 10 hr. Isoflavonoids in the EtOAc extract of cultures from 11 flasks incubated for 10 hr were initially divided into 3 fractions by multiple development silica gel PTLC (CM = 10:1, × 3). The upper band (unchanged substrate) was re-chromatographed in BE (3:1) to give 20.2 mg of **2** (R_f 0.51). Elution of the middle band yielded, after concn, 7.4 mg of **3** as pale yellow needles. A further 2.3 mg of **3** was also obtained from the mother liquor by re-PTLC in BE (1:1, R_f 0.64). The lowest running isoflavone (17.4 mg) was further purified by re-PTLC in CEAM (15:5:5:1) to yield 15 mg of a pale yellow gum (**4**, R_f 0.26).

Physicochemical properties of the metabolites. (−)−**3**. Pale yellow needles from EtOAc, mp 173–175°. $UV_{(365\,nm}$ fluorescence, dark purple. Gibbs test: (+), rapid, purple-blue. $[\alpha]_{405}^{23}$ − 6.0° (MeOH; *c* 0.127). MS *m/z* (rel. int.): 385 $[M+1]^+$ (7), 384 $[M]^+$ (31), 341 (22), 325 (9), 314 (23), 313 $[M-71]^+$ (100), 312 (49), 311 (8), 295 (13), 207 (6), 180 (7), 179 [RDA fragment of A-ring with CH_2 alkyl remnant] (63), 164 (8), 149 (12), 135 (5), 134 [RDA fragment of B-ring] (11), 121 (6), 69 (9). UV λ_{max}^{MeOH} nm (ε): 217sh (27 300), 263 (34 200). 330sh (*br*, 4700); + NaOMe, 245sh, 255sh, 262, 271sh, 318sh; + $AlCl_3$, 235sh, 273.5, 313sh, 384 (br); + NaOAc, unchanged. ^{1}H NMR data, see Table 1.

4. Pale yellow gum. $UV_{(365\,nm)}$ fluorescence, dark purple. Gibbs test: (+), rapid, purple-blue. $[\alpha]_D^{23}$ − 5.8° and $[\alpha]_{405}^{23}$ − 19° (MeOH; *c* = 0.123). MS *m/z* (rel. int.): 403 $[M+1]^+$, (13), 402 $[M]^+$ (24), 344 (19), 343 $[M-59]^+$ (59), 314 (33), 313 $[M-89]^+$ (100), 312 (9), 301 (14), 295 (10), 209 (9), 181 (8), 180 (9), 179 (40), 153 (10), 149 (8), 134 (11), 123 (8), 69 (10), 59 (15). UV λ_{max}^{MeOH} nm (ε): 220sh (25 900), 264.5 (34 100), 330sh (br, 4500); + NaOMe, 254sh, 264, 273sh; + $AlCl_3$, 235sh, 274, 315sh, 380 (br); + NaOAc, unchanged. ^{1}H NMR assignments are shown in Table 1. Irradiation at δ3.64 (2″-H) caused two signal changes: one, δ3.39 *d* → singlet, and the other, δ2.97 (*d* + *d*) → singlet. Two signals (a singlet at δ3.34 and a doublet at δ3.39) disappeared upon addition of one drop of D_2O to the acetone-d_6 used as the ^{1}H NMR solvent.

Acid-catalysed hydrolysis of (−)-**3**. The acid-catalysed hydrolysis of **3** was undertaken using the procedure ref. [23] to prenyl epoxide derivatives of coumarins. Laevorotatory **3** (3.5 mg) was first dissolved in aq. 1,4-dioxane (95%, 1 ml), and 50 μl of 2 M H_2SO_4 was then added to the stirred solution. After stirring for a further 20 min at room temp., the reaction mixture was dild with aq. 5% $NaHCO_3$ and shaken with EtOAc (70 ml). The EtOAc extract was then washed with brine before being chromatographed (silica gel PTLC in CEAM = 15:5:5:1) to give **4** (R_f 0.26) as the major reaction product. A small quantity of unchanged starting material was also recovered (R_f 0.73). Compound **4** (2.3 mg) produced chemically from **3** was indistinguishable from metabolite **4** by TLC and MS comparison. Both compounds gave the same Gibbs test colour, and exhibited an identical fluorescence on chromatograms viewed under $UV_{(365\,nm)}$ light. Physico-chemical properties recorded for the major hydrolysate product were as follows: MS *m/z* (rel. int.): 402 $[M]^+$ (18), 344 (22), 341 (69), 314 (33), 313 (100), 301 (11), 179 (39), 134 (11). $[\alpha]_{405}^{23} - 18°$ (MeOH; *c* 0.077).

CD determination of the osmate ester-pyridine complex of **4**. Dry **4** (2.5 μmol) was dissolved in a mixture of CH_2Cl_2 (118 μl) + pyridine (4 μl) and added to a solution of OsO_4 in CH_2Cl_2 (0.69 mg/9.2 μl) [1, 22]. After being kept at 23° for 3 hr, the reaction mixture was diluted with more CH_2Cl_2 to give a final vol. of 2.8 ml. The CD spectrum of this solution was recorded at 23° over the range 350–650 nm using a Model J-20A Automatic Recording Spectropolarimeter (Japan Spectroscopic Co. Ltd.): $[\theta]_{470\,nm} - 1260$ (*S*-configuration [22]).

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