

# Metabolism of the Prenylated Pterocarpan Edunol by *Aspergillus flavus*

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When incubated in liquid culture with *Aspergillus flavus*, the prenylated pterocarpan (–)-edunol [2-(3,3-dimethylallyl)-3-hydroxy-8,9-methylenedioxypterocarpan (**1**)] was converted into a dihydrofurano-pterocarpan (**2**), a dihydropyrano-pterocarpan (**3**), and a 2,3-dihydro-dihydroxyprenyl-substituted pterocarpan (**4**).

## Introduction

In our previous papers, we reported that isoflavones with a 3,3-dimethylallyl (prenyl) substituent at C-6, C-8 (ring A) or C-3' (ring B) were variously metabolized by the fungus *Aspergillus flavus* to give hydrates (luteone [1] and wighteone [2]), and derivatives possessing dihydrofurano, dihydropyrano or 2,3-dihydrodihydroxyprenyl side-attachments (luteone [1], wighteone [2], 2,3-dehydrokievitone [3], licoisoflavone A [4], and 2'-hydroxylupalbigenin [5]). Studies involving the plant pathogenic fungus *Fusarium oxysporum* f. sp. *phaseoli* have shown that two other prenylated isoflavonoids, kievitone [5,7,2',4'-tetrahydroxy-8-(3,3-dimethylallyl)isoflavanone] and phaseollidin [3,9-dihydroxy-10-(3,3-dimethylallyl)pterocarpan] are also metabolized *in vitro* to give the corresponding hydrates [6, 7]. In kievitone and phaseollidin, the prenyl groups are located on different aromatic rings, and their hydration by *F. oxysporum* f. sp. *phaseoli* suggests that the enzyme responsible (kievitone hydratase [8]) is relatively non-specific in its action, or the fungus contains two hydratases differing in the substrate specificity [9].

In contrast, *A. flavus* exhibits a higher degree of substrate preference bringing about hydration of prenyl groups at C-6 on ring A of isoflavones (luteone and wighteone [1, 2], but not those located at C-8 (also ring A; 2,3-dehydrokievitone [3]) or C-3' (ring B; licoisoflavone A and 2'-hydroxylupalbigenin [4,

5]). The present study involving (–)-edunol [2-(3,3-dimethylallyl)-3-hydroxy-8,9-methylenedioxypterocarpan (**1**)], a fungitoxic [10] isoflavonoid from the root bark of *Neorautanenia edulis* (Leguminosae) [11], was undertaken to determine if *A. flavus* could: a) metabolize an isoflavonoid of a type different from the isoflavones previously tested, and b) convert the prenyl group at C-2 ( $\equiv$  C-6 of isoflavones) into a hydrated sidechain analogous with that encountered in the hydrates of luteone and wighteone [1, 2].

## Results and Discussion

When shaken for 4 days (25 °C) in a liquid medium with *A. flavus*, (–)-edunol (**1**) was gradually metabolized to give three laevorotatory products designated ED-AF-1, ED-AF-2 (both  $M^+$  368), and ED-AF-3 ( $M^+$  386). These compounds, together with unchanged edunol ( $M^+$  352), were extracted from the medium with EtOAc, and then were separated by preparative Si gel TLC (PTLC) as outlined in the Experimental section. Their characterization as pterocarpan **2–4** is described in this report. Yields and comparative  $R_F$  values for each metabolite, and for the substrate, are shown in Table I. Surprisingly, no evidence was obtained to indicate that edunol could be converted to a hydrate derivative (expected  $M^+$  370) despite the fact that *A. flavus* has been found to readily hydrate the isoflavones luteone and wighteone [1, 2], each of which is prenylated at the position (C-6) equivalent to C-2 in **1**.

The UV (MeOH) spectrum of metabolite ED-AF-1 ( $M^+$  368; = substrate + [O]) was unaffected by NaOMe indicating the absence of a phenolic OH

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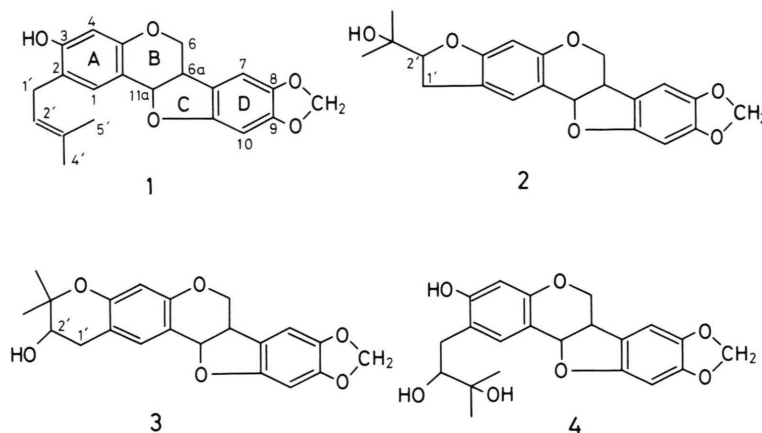


Table I. Chromatographic properties and yields of edunol and its metabolites.

Compound	$R_F$ (TLC) value <sup>a</sup>		Yield <sup>b</sup>	
	CM (25:1)	CAAm (35:30:1)	[mg]	[%]
Edunol ( <b>1</b> )	0.86	0.82	7.4	24.7
ED-AF-1 ( <b>2</b> )	0.85	0.78	3.0	9.6
ED-AF-2 ( <b>3</b> )	0.80	0.78	8.4	26.8
ED-AF-3 ( <b>4</b> )	0.26	0.27	5.9	17.9

<sup>a</sup> Solvent system abbrev., CM =  $\text{CHCl}_3$ -MeOH; CAAm =  $\text{CHCl}_3$ -acetone-conc. aqueous  $\text{NH}_3$ .

<sup>b</sup> Yield in mg from 30 mg of substrate (**1**); % yield is on a molar basis.

group (*cf.* the alkaline spectrum of **1** with a C-3 hydroxyl substituent). This observation, and the difference of 16 atomic mass units between the molecular weight of **1** and ED-AF-1, can be explained by the fungal-mediated formation of an ether ring side-attachment involving the substrate prenyl (C-2) and the *ortho* (C-3)-located OH group.

In the  $^1\text{H}$  NMR spectrum of ED-AF-1, this side-attachment afforded a set of signals [ $\delta$  1.21 and 1.24 (both 3H, two s, 4'- and 5'- $\text{H}_3$ ), 3.07 and 3.26 (both 1H, two dd,  $J$  = 16.3 & 9.3 Hz, and 16.3 & 8.3 Hz, 1'- $\text{H}_a$  and 1'- $\text{H}_b$ ), and 4.64 (1H, dd,  $J$  = 9.8 & 8.3 Hz, 2'-H)] with chemical shift values closely resembling those given by the 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran substituent previously found in several isoflavones including luteone metabolite BC-1 [1] and lupinisoflavones B-F [12]. Prominent MS fragments at  $m/z$  309 ( $M^+$  - 59; 11%) and 59 (24%) were also consistent with the presence of such a side-

attachment [1].  $^1\text{H}$  NMR signals attributable to the aromatic (A/D) and heterocyclic (B/C) ring protons, and the  $\text{O}-\text{CH}_2-\text{O}$  group, of edunol (Table II) were similarly evident in the spectrum of ED-AF-1, thereby permitting the metabolite to be formulated as shown in **2**. This structure has already been assigned to "neoplanol", a racemic dihydrofurano-pterocarp produced by chemical modification of edunol [13], but not as yet reported to be a natural product. MS and  $^1\text{H}$  NMR data obtained for ED-AF-1 were in good agreement with those published [13] for synthetic "neoplanol" (see Table II, and the Experimental section for comparative details).

The  $^1\text{H}$  NMR spectrum of ED-AF-2 ( $M^+$  368; = substrate + [O]) clearly revealed that this major, non-phenolic *Aspergillus* metabolite differed from **2** only with respect to the nature of the A-ring side-attachment (Table II). Thus, instead of a dihydrofurano substituent, a set of aliphatic proton signals at  $\delta$  1.25 and 1.33 (both 3H, two s, 4'- and 5'- $\text{H}_3$ ), 2.62 and 3.01 (both 1H, two dd,  $J$  = 16.5 & 7.3 Hz, and 16.5 & 5.4 Hz, 1'- $\text{H}_a$  and 1'- $\text{H}_b$ ) and 3.79 (1H, ddd,  $J$  = 7.3, 5.4 & 5.1 Hz, 2'-H coupled to 2'-OH with  $J$  = 5.1 Hz) defined the presence of a 2,3-dihydro-3-hydroxy-2,2-dimethylpyrano side-attachment as in luteone metabolite BC-2 [1], 2,3-dehydrokievitone metabolite DK-M2 [3], and licoisoflavone A metabolites M-1-1 and M-3-1 [4]. As with these four metabolites, the MS of ED-AF-2 afforded a characteristic fragment at  $M^+$  - 71 ( $m/z$  297; 98%) [1, 3, 4]. Metabolite ED-AF-2 must therefore have structure **3**. Neorautanol from *Neorautanenia amboensis* has also been formulated as **3**. MS and  $^1\text{H}$  NMR data

Table II.  $^1\text{H}$  NMR data ( $\delta$  values) for edunol and its *Aspergillus flavus* metabolites<sup>a</sup>

Compound Proton	Edunol ( <b>1</b> )	ED-AF-1 ( <b>2</b> ) <sup>b</sup>	ED-AF-2 ( <b>3</b> ) <sup>b</sup>	ED-AF-3 ( <b>4</b> )
1-H	7.16 s	7.22 s	7.15 s	7.22 s
4-H	6.38 s	6.21 s	6.23 s	6.34 s
7-H	6.88 s	6.89 s	6.90 s	6.89 s
10-H	6.40 s	6.40 s	6.39 s	6.40 s
6-H <sub>eq</sub>	4.24 m	4.27 m	4.26 m	4.27 m
6-H <sub>ax</sub>	3.55 m	3.58 m	3.60 m	3.57 m
6a-H	(2H)	(2H)	(2H)	(2H)
11a-H	5.46 br. d $J=6.4$	5.50 br. d $J=6.4$	5.49 br. d $J=6.1$	5.47 br. d $J=6.1$
O-CH <sub>2</sub> -O	5.90 d $J=1.0$	5.91 d $J=1.0$	5.91 d $J=1.0$	5.91 d $J=1.0$
	5.93 d $J=1.0$	5.94 d $J=1.0$	5.94 d $J=1.0$	5.93 d $J=1.0$
1'-H <sub>a</sub>	3.29 br. d (2H) $J=7.3$	3.07 dd $J=16.3, 9.3$	2.69 dd $J=16.6, 7.3$	2.62 dd $J=14.5, 9.5$
1'-H <sub>b</sub>		3.26 dd $J=16.3, 8.3$	3.01 dd $J=16.6, 5.4$	2.93 dd $J=14.5, 2.3$
2'-H	5.35 br. t $J=7.3$	4.64 dd $J=9.3, 8.3$	3.79 ddd $J=7.3, 5.4, 5.1$	3.64 dd $J=9.4, 2.3$
4'-CH <sub>3</sub>	1.73 s (3H)	1.21 s (3H)	1.25 s (3H)	1.25 s (3H)
5'-CH <sub>3</sub>	1.74 s (3H)	1.24 s (3H)	1.33 s (3H)	1.27 s (3H)
2'-OH	—	—	4.23 d $J=5.1$	—
3'-OH	—	3.62 s	—	—

<sup>a</sup> All spectra were determined in acetone- $d_6$  at 100 MHz (TMS reference). Coupling constants ( $J$ ) are in Hz. For multiplets, the  $\delta$  value indicates the centre of the signal.

<sup>b</sup>  $^1\text{H}$  NMR chemical shift values for synthetic "neoplanol" (= **2**) and natural neorautanol (= **3**) in  $\text{CDCl}_3$  (80 MHz) are reported in ref. [13].

obtained for ED-AF-2 agreed reasonably with those of neorautanol [13].

The third metabolite, ED-AF-3 ( $M^+$  386; = substrate +  $2 \times [\text{OH}]$ ), ran well below **2** and **3** on thin-layer chromatograms developed in both CM and CAAM (Table I). This increased polarity relative to the other *Aspergillus* metabolites, coupled with the detection of an intense MS ion at  $M^+ - 89$  ( $m/z$  297; 100%), suggested that ED-AF-3 contained a 2,3-dihydrodihydroxyphenyl side-chain [1, 3, 4]. An underivatized C-3 OH group was apparent from the UV (MeOH + NaOMe) maximum at 251 nm [14].

Structure **4** for ED-AF-3 was confirmed by the  $^1\text{H}$  NMR spectrum which exhibited signals typical of a 1,2-glycol type side-chain at  $\delta$  1.25 and 1.27 (both 3H, two s, 4'- and 5'-H<sub>3</sub>), 2.62 and 2.93 (both 1H, two dd,  $J=14.5$  & 9.4 Hz, and 14.5 & 2.3 Hz, 1'-H<sub>a</sub> and 1'-H<sub>b</sub>) and 3.64 (1H, dd,  $J=9.4$  & 2.3 Hz, 2'-H). In all other respects, the  $^1\text{H}$  NMR spectrum closely resembled that of edunol (Table II). Apart from its appearance in ED-AF-3, the 2,3-dihydrodihydroxyphenyl (2,3-dihydroxy-3-methylbutyl) side-chain has also been encountered in luteone glycol [1], wight-eone glycol [2], 2,3-dehydrokievitone glycol [3] and

licoisoflavone A glycol [4], all of which are produced by *A. flavus* from the corresponding isoflavone substrates.

## Experimental

General procedures (e.g. silica gel PTLC, and m.p., UV, MS and  $^1\text{H}$  NMR measurements) were undertaken using the equipment and conditions previously described [4, 12]. The substrate pterocarpan (**1**) was isolated from the shredded root bark of *Neorautanenia edulis* [11].

### Edunol (**1**) [2-(3,3-dimethylallyl)-3-hydroxy-8,9-methylenedioxypterocarpan]

Colourless needles, m.p. 151–152 °C;  $[\alpha]_{\text{D}}^{23} - 325^\circ$  ( $c = 0.088$ , MeOH). UV:  $\lambda_{\text{max}}$ , nm: MeOH 232 sh, 288 sh, 294, 310; + NaOMe, 251, 304. MS (rel. int. %):  $m/z$  353 ( $\text{M}^+ + 1$ ; 25), 352 ( $\text{M}^+$ ; 100), 298 (14), 297 ( $\text{M}^+ - 55$ ; 71), 296 (31), 176 (12), 175 (21), 148 (16), 147 (13), 71 (12), 69 (12), 57 (21).

### Metabolic experiments

*Aspergillus flavus* (isolate AHU 7049) was cultured for 4 days in a shaking liquid medium consisting of glucose (5 g), peptone (1 g), yeast extract (0.1 g) and  $\text{H}_2\text{O}$  (100 ml). A solution of **1** (5 mg in 1 ml of EtOH) was then added, and after a further 4 days incubation the metabolites, and any remaining substrate, were extracted from the medium with EtOAc (see ref. [1] for exact details).

### Isolation and purification of edunol metabolites

An EtOAc extract of the combined *Aspergillus* culture medium (600 ml, initially containing 30 mg of **1**) from 6 flasks was washed with 5% aqueous  $\text{NaHCO}_3$ , and then with a saturated solution of NaCl. After removal of the EtOAc *in vacuo* (40 °C), the residue was chromatographed (Si gel preparative TLC) in  $\text{CHCl}_3$ -acetone-conc. aqueous  $\text{NH}_3$  (CAAm; 35:30:1) to afford unchanged edunol (upper band), a mixture of ED-AF-1 and ED-AF-2 (middle band), and ED-AF-3 (lower band). See Table I for  $R_F$  values. After elution with EtOAc, edunol and ED-AF-3 were rechromatographed in  $\text{CHCl}_3$ -MeOH (CM; 25:1) to give the pure pterocar-

pans. After concentration, the eluate of the middle band (CAAm chromatogram) deposited colourless plates of ED-AF-2. The mother liquor was then chromatographed (Si gel PTLC in CM,  $\times 3$ ) to afford ED-AF-1 (upper zone) and a further quantity of ED-AF-2 (lower zone). The yields of each metabolite are given in Table I.

### Metabolite ED-AF-1 (**2**; = "neopranol" [13])

Colourless fine rods, m.p. 204–205 °C (subliming at  $>200^\circ\text{C}$ );  $[\alpha]_{\text{D}}^{23} - 340^\circ$  ( $c = 0.096$ , MeOH). UV:  $\lambda_{\text{max}}$ , nm: MeOH 232 sh, 291 sh, 296 sh, 302, 308 sh; + NaOMe, no change. MS (rel. int. %):  $m/z$  369 ( $\text{M}^+ + 1$ ; 24), 368 ( $\text{M}^+$ ; 100), 335 (16), 310 (18), 309 ( $\text{M}^+ - 59$ , 11), 297 (27), 296 (12), 175 (18), 162 (25), 160 (13), 147 (13), 71 (16), 59 (24), 57 (29). MS data published for synthetic "neopranol" [13] are:  $m/z$  368 ( $\text{M}^+$ ; 100), 353 (2), 335 (14), 310 (16), 297 (25), 175 (19), 162 (29), 160 (10), 151 (12), 148 (15).

### Metabolite ED-AF-2 (**3**; = neorautanol [13])

Colourless plates, m.p. 207–209 °C (subliming at  $>195^\circ\text{C}$ ; neorautanol [13], m.p. 93–95 °C);  $[\alpha]_{\text{D}}^{23} - 271^\circ$  ( $c = 0.136$ , MeOH). UV:  $\lambda_{\text{max}}$ , nm: MeOH 232 sh, 285 sh, 289 sh, 295, 310; + NaOMe, no change. MS (rel. int. %):  $m/z$  369 ( $\text{M}^+ + 1$ ; 24), 368 ( $\text{M}^+$ ; 100), 335 (8), 298 (22), 297 ( $\text{M}^+ - 71$ ; 98), 296 (27), 267 (9), 175 (9), 162 (17), 85 (8), 71 (16), 69 (12), 57 (27).

### Metabolite ED-AF-3 (**4**)

Colourless fine rods, m.p. 195–197 °C (subliming at  $>185^\circ\text{C}$ );  $[\alpha]_{\text{D}}^{23} - 277^\circ$  ( $c = 0.188$ , MeOH). UV:  $\lambda_{\text{max}}$ , nm: MeOH 227 sh, 281 sh, 287 sh, 291.5, 310.5; + NaOMe, 251, 303.5. MS (rel. int. %):  $m/z$  387 ( $\text{M}^+ + 1$ ; 22), 386 ( $\text{M}^+$ ; 92), 368 ( $\text{M}^+ - 18$ ; 34), 298 (23), 297 ( $\text{M}^+ - 89$ ; 100), 296 (20), 175 (11), 162 (15), 151 (14), 85 (11), 71 (21), 69 (16), 59 (13), 57 (38).

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