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TWO CINNAMIC ALLENIC ETHERS FROM THE FUNGUS *CLITOCYBE EUCALYPTORUM**

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Key Word Index—*Clitocybe eucalyptorum*; Basidiomycetes; allenic ether; *cis*- and *trans*-cinnamates; eucalyptenes A and B.

Abstract—The structure of eucalyptene A, a fungal metabolite isolated from *Clitocybe eucalyptorum*, has been determined to be 4′-(2″,3″-butadienyloxy) *trans*-cinnamic acid methyl ester on the basis of ^1H and ^{13}C NMR evidence and chemical synthesis. Minor amounts of its *cis* isomer, eucalyptene B, were also isolated.

INTRODUCTION

During screening of the genus *Clitocybe* for secondary metabolites, we reported the isolation of a large number of protoilludane sesquiterpenes, viz. melledonals D and E from *C. elegans* [2], candicansol and 3-*epi*-illudol from *C. candicans* [3], and illudines A and B [4], illudalenol [4] and illudosin [5] from *C. illudens* (*O. olearius*). Further investigation of the *Clitocybe* genus led to the isolation from a strain of *C. eucalyptorum*, when grown in MPGA (malt, peptone, glucose and agar) medium, of a novel allenic ether of *p*-hydroxy-*trans*-cinnamic acid methyl ester, named eucalyptene A (**3**), together with some minor amounts of its *cis* isomer, eucalyptene B (**4**).

RESULTS AND DISCUSSION

Eucalyptene A (**3**) was isolated as a solid and its molecular formula $C_{14}H_{14}O_3$ established by EI mass spectrometry, the peaks at *m/z* 215 $[M-Me]^+$ and 178 $[M-C_4H_4]^+$ indicating the loss of a methyl group and of a four-carbon fragment. Absorptions at 1965 and 1715 cm^{-1} in the IR spectrum suggested the presence of allenic and conjugated ester groups. The ^1H NMR spectrum in chloroform-*d* (Table 1) showed the presence of a singlet at δ3.79 due to a methoxyl group and AX and AA′XX′ spin systems attributable to the protons of a *trans*-disubstituted double bond ($^3J_{2,3}$ = 16.0 Hz) and of a *para*-disubstituted phenyl moiety. In addition, it displayed an A_2MX_2 spin system due to the C-1″, C-2″ and C-4″ protons. The broad-band ^1H-decoupled and the fully ^1H-coupled ^{13}C NMR spectra confirmed the presence of 14 carbon atoms (1 × Me, 2 × CH_2, 7 × CH and 4 × C). Four carbons were assigned to the –C(3)H=C(2)H–C(1)O_2C(4)H_3 methyl acrylate moiety, six carbons, C-1′ to C-6′, to the aromatic ring, with the remaining four carbons, C-1″ to C-4″, being attributed to a 2,3-butadienyloxy group. In fact, the chemical-shift values of δ 86.7, 209.6 and 76.8 observed for C-2″, C-3″ and C-4″ [6], together with the one-bond ^1H–^{13}C couplings of 167 and 169 Hz exhibited by C-2″ and C-4″ [7], indicate that they are part of a –C(2″)H=C(3″)=C(4″)H_2 moiety, while the presence of ^1H–^1H couplings of 6.8 and 2.5 Hz between the 1″-methylene protons, which were correlated with the oxygen-bearing carbon at δ65.9, and H-2″ and H'_2-4 defines the linkage between C-1″ and C-2″.

Definitive evidence for the proposed structure **3** followed from the synthesis of eucalyptene A shown in Scheme 1. This was accomplished by reacting 1-chloro-4-hydroxybut-2-yne [8] with *p*-hydroxy-*trans*-cinnamic acid methyl ester as the sodium salt in dry tetrahydrofuran. Chlorination with thionyl chloride of the resulting acetylenic ether **1** gave the chloro derivative **2** [9] which was reduced to eucalyptene A (**3**) with zinc–copper complex in methanol [10]. Finally, compound **3** on irradiation in isopropyl alcohol with 300 nm light yielded the *cis*-isomer **4**, as evidenced by the presence in its ^1H NMR spectrum (Table 1) of a ^1H–^1H coupling of 12.2 Hz between the olefinic protons typical of a *cis*-geometry.

The spectroscopic properties of the synthesized compounds **3** and **4**, i.e. ^1H NMR, IR and UV spectral data, were identical to those of the natural ones.

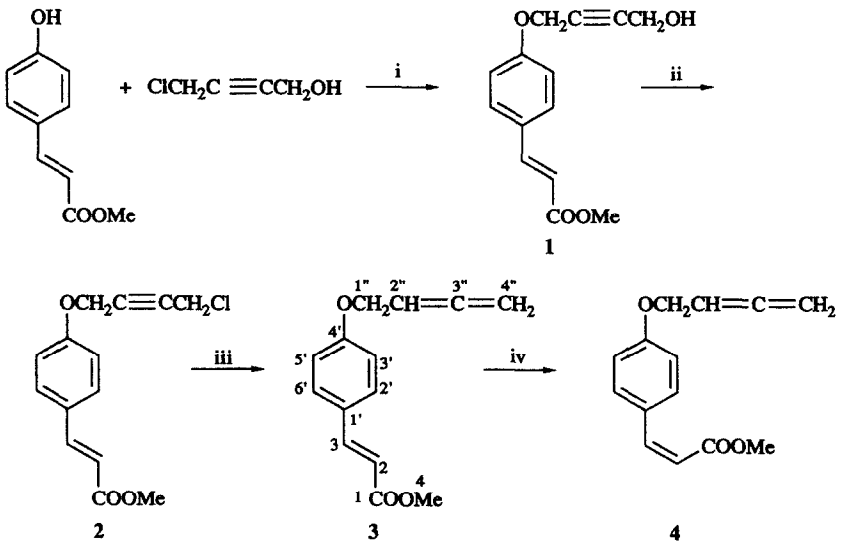
Allenic functions in basidiomycetous fungi are formed by isomerization of acetylenes by isomerases; in particular, the genus *Clitocybe* produces a series of polyacetylenes active as inhibitors of fungi and of seed germination [11, 12]. Cinnamic acids are widely present in higher plants and fungi, and are active as growth inhibitors of plants and spore germination [13]. A similar allenic ether, chestersiene, has been isolated from the fungus *Hypoxylon chestersii* and it is the only other report of the

*Part 40 in the series 'Secondary Mould Metabolites'. For Part 39 see ref. [1].

Table 1. ^{13}C and ^{1}H NMR spectral data of compounds 3 and 4 (in $CDCl_3$)

	3			4	
Atom	δ_C	δ_H	J (H, H) (Hz)	δ_H	J (H, H) (Hz)
1	167.8				
2	115.4 (162)*	6.31 *d*	16.0	5.83 *d*	12.2
3	144.5 (155)	7.65 *dtt*	16.0, 0.6, 0.4	6.85 *br d*	12.2
4	51.6 (147)	3.79 *s*		3.73 *s*	
1′	129.7				
2′	130.3 (158)	7.47 *m*		7.69 *m*	
3′	115.2 (161)	6.91 *m*		6.90 *m*	
4′	160.2				
5′	115.2 (161)	6.91 *m*		6.90 *m*	
6′	130.3 (158)	7.47 *m*		7.69 *m*	
1″	65.9 (148)	4.60 *dt*	6.8, 2.5	4.61 *dt*	6.8, 2.5
2″	86.7 (167)	5.38 *tt*	6.8, 6.6	5.39 *tt*	6.8, 6.6
3″	209.6				
4″	76.8 (169)	4.89 *dt*	6.6, 2.5	4.88 *dt*	6.6, 2.5

*Values in parentheses are one-bond $^{1}H-^{13}C$ coupling constants in Hz.



Scheme 1. Synthesis of eucalyptene A (3). Reagents: (i) NaH–THF; (ii) $SOCl_2$–THF; (iii) Cu, Zn–MeOH; (iv) hν (300 nm)–2-propanol.

natural occurrence of an aromatic allenic ether [14]. Eucalyptenes A (3) and B (4) exhibit antifungal activity, revealed by means of bioautography on *Cladosporium cladosporioides* for amounts as low as 50 μg.

EXPERIMENTAL

Mps: uncorr.. Flash CC was performed on silica gel (0.04–0.06 mm), TLC on HF_{254} silica gel. MS were recorded at 60 eV. ^{1}H NMR were recorded at 300 MHz, ^{13}C NMR at 63 MHz. Chemical shifts are in ppm (δ) from TMS as int. standard. The apparatus used for irradiation of 3 was a Rayonet RPR-100 (Southern New England UV Co.) equipped with 16 fluorescent lamps irradiating at 300 nm.

Cultivation of fungus and isolation of eucalyptene. The CBS 433.72 strain of *C. eucalyptorum* was inoculated in 40 Roux flasks containing MPGA (100 ml) (malt ext.–peptone–glucose–agar, 20:5:20:15 $g l^{-1}$) with a mycelium suspension. After 3 weeks, flasks were extracted with EtOAc containing 1% MeOH, the combined extracts dried (Na_2SO_4) and evapd to give a crude extract (1.1 g). This mixt. was chromatographed on a silica gel column using hexane–EtOAc (4:1) and purified further by prep. TLC (PLC) with hexane–EtOAc (9:1) to yield eucalyptenes A (3) (150 mg) and B (4) (20 mg).

4′-(2″,3″-*butadienyloxy*)trans-*Cinnamic acid methyl ester* (3) (*eucalyptene A*). Solid, mp 68° (from hexane). (Found: C, 72.88; H, 6.10. $C_{14}H_{14}O_3$ requires: C, 73.02; H, 6.13%.) EIMS (rel. int.) *m/z*: 230 $[M]^+$ (45), 215 (15), 192

(72), 178 (100) and 149 (69); CIMS (isobutane) *m/z*: 231 $[MH]^+$. UV $\lambda_{max}^{95\%EtOH}$ nm: 204, 220 and 302 (ε 12050, 10950 and 19060). ^1H and ^{13}C NMR in Table 1.

*4'-(2'',3''-butadienyloxy)*cis-*Cinnamic acid methyl ester* (**4**) (*eucalyptene B*). Oil. EIMS (rel. int.) *m/z*: 230 $[M]^+$ (50), 215 (10), 192 (70), 178 (100) and 149 (70). IR $\nu_{max}^{Liquid\ film}$ cm^{-1}: 1965 (allene) and 1715 (ester CO).

*4'-(2''-butynyloxy-4''-hydroxy)*trans-*Cinnamic acid methyl ester* (**1**). To a stirred suspension of NaH (80%, 440 mg, 14.6 mmol) in dry THF (10 ml), a soln of *p*-hydroxycinnamic acid Me ester (1.3 g, 7.3 mmol) in 5 ml of dry THF was added dropwise. The suspension was then refluxed for 1 hr. After cooling, 1-chloro-4-hydroxybut-2-yne (760 mg, 7.3 mmol) dissolved in dry THF was added dropwise. The mixt. was stirred under reflux for 48 hr. After cooling, H_2O was added and extraction was performed (Et_2O, 2 × 10 ml). The combined Et_2O extracts were dried (Na_2SO_4), the solvent evapd and the resulting solid was chromatographed on a flash silica gel column using hexane–EtOAc (2:1). Compound **1** was obtained as a solid (1.1 g, 60%), mp 98° (from EtOH). EIMS *m/z*: 246 $[M]^+$, 228, 203 and 178. ^1H NMR ($CDCl_3$): δ1.90 (1H, *br s*, OH), 3.80 (3H, *s*, OMe), 4.30 (2H, *br s*, H_2-4''), 4.73 (2H, *br s*, H_2-1''), 6.27 (1H, *d*, *J* = 15.8 Hz, H-2), 6.93 (2H, *m*, H-3' and H-5'), 7.45 (2H, *m*, H-2' and H-6') and 7.60 (1H, *d*, *J* = 15.8 Hz, H-3).

*4'-(2''-butynyloxy-4''-chloro)*trans-*Cinnamic acid methyl ester* (**2**). Compound **1** (0.5 g, 2 mmol) was dissolved in dry THF (10 ml) and dry pyridine (0.2 ml) added under stirring together with thionyl chloride (0.12 g, 1 mmol) dissolved in dry THF (2 ml). The mixt. was refluxed for 12 hr, ice-H_2O added and extraction was performed (Et_2O, 2 × 10 ml). The combined Et_2O extracts were dried (Na_2SO_4) and the solvent evapd. The crude compound was purified by chromatography (hexane–EtOAc, 2:1) to give **2** as a solid (0.5 g, 95%), mp 58°. EIMS *m/z*: 264 $[M]^+$, 229, 197, 187, 178 and 147. ^1H NMR ($CDCl_3$): 3.79 (3H, *s*, OMe), 4.17 (2H, *t*, *J* = 2.1 Hz, H_2-4''), 4.78 (2H, *t*, *J* = 2.1 Hz, H_2-1''), 6.34 (1H, *d*, *J* = 15.9 Hz, H-2), 6.96 (2H, *m*, H-3' and H-5'), 7.50 (2H, *m*, H-2' and H-6') and 7.65 (1H, *d*, *J* = 15.9 Hz, H-3).

Eucalyptene A (**3**). The chloro derivative **2** (0.2 g) was dissolved in dry MeOH (3 ml) and added under stirring to a suspension of Cu (0.1 g) and Zn (0.1 g) powders and the mixt. refluxed for 24 hr. The soln was then cooled, centrifuged and the solvent evapd under vacuum. The residue was recrystallized from hexane yielding a compound identical to the natural one.

Eucalyptene B (**4**). Eucalyptene A (**3**) (30 mg) on irradiation in isopropyl alcohol (20 ml) with 300 nm light yielded the *cis*-isomer, eucalyptene B (**4**), as an oil in a 60% yield.

Antifungal tests. These expts were performed by means of bioautography. Pure metabolites were loaded in the amounts of 50, 100 and 200 μg on a TLC plate, which was, after development, sprayed with a conidial suspension of *C. cladosporioides* in Czapek Dox Broth. The plate was then incubated in a moist chamber and antifungal activity was evidenced by the appearance of a white spot, corresponding to the position of the active metabolite, surrounded by a grey-black fungal growth all over the plate.

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