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(−)-7-HYDROXYCALAMENENE, A PHYTOALEXIN FROM *TILIA EUROPEA*

RAYMOND S. BURDEN and MALCOLM S. KEMP

Long Ashton Research Station, University of Bristol, Long Ashton, Bristol, BS18 9AF, U.K.

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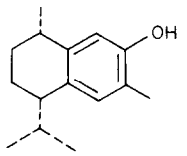
Key Word Index—*Tilia europea*; Tiliaceae; European Lime; *Ganoderma applanatum*; fungal infection; sesquiterpene; (−)-7-hydroxycalamenene.

Abstract—The antifungal sesquiterpene (−)-7-hydroxycalamenene has been isolated and identified in *Tilia europea* (European Lime) infected with the fungus *Ganoderma applanatum*. It was found in the narrow pigmented boundary zone between healthy and infected wood.

INTRODUCTION

Post-infectional antifungal compounds (phytoalexins) have been isolated and identified from infected tissues of many herbaceous plants [1] but little is known about their distribution and importance in trees. Here we report the accumulation of an antifungal phenolic sesquiterpene (−)-7-hydroxycalamenene (**1**) in a diseased mature specimen of European Lime (*Tilia europea*).



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RESULTS AND DISCUSSION

The single tree studied in the present investigation had large fruiting bodies of the fungus *Ganoderma applanatum* growing out from the trunk and, when the tree was felled and sectioned, the inner core was found to be extensively decayed. Samples of wood were taken from the decayed centre, the healthy sapwood in the outer region of the trunk and also from the narrow (*ca* 1 mm) darkly pigmented boundary which occurred between healthy and infected wood.

Extracts of all three samples were bioassayed [2] against *Cladosporium cucumerinum* on TLC plates. Only the extract of interface wood gave a large area of inhibition. The substance responsible was isolated by chromatography and identified as (−)-(1*S*,4*S*)-7-hydroxycalamenene from a comparison of mass, ^{1}H NMR, UV and mass spectra and $[\alpha]_D$ with literature data [3]. It was also characterized as the 3,5-dinitrobenzoate.

(−)-7-Hydroxycalamenene was first isolated [3] from the heartwood of *Ulmus thomasii* (Ulmaceae) and has subsequently been found in the heartwood of other healthy elm species [4]. The (+)-enantiomer has been isolated from *Eremophila drummondii* (Myoporaceae) and its structure determined by X-ray crystallography [5].

There has been no reported investigation of the antimicrobial properties of 7-hydroxycalamenene but in our experiments as little as 0.5 μg gave a well-defined inhibition zone on a TLC plate sprayed with *Cladosporium cucumerinum*. This observation, taken together with the high concentration (*ca* 1% fr. wt) detected at the disease boundary, would suggest that the phenol may retard the growth of the invading pathogen. However, the extensive decay observed would suggest that it is only partially effective and that the formation of the necrotic interface zone is a dynamic process, i.e. the decay fungus may be able to degrade the phenol but adjacent areas of sapwood then react to produce more [6]. Clearly, further studies are needed to assess the role of 7-hydroxycalamenene and similar compounds in the natural defence of trees against fungal pathogens.

EXPERIMENTAL

A diseased European Lime (*ca* 60 years old) was felled and cut into sections. Samples (2 g) of healthy wood, diseased wood and pigmented interface wood were planed and the shavings soaked in EtOH (30 ml) for 7 days. The extracts were filtered, evaporated and the residues redissolved in EtOH (2 ml). Aliquots (20 μl) were then applied to Merck Si gel F254 TLC plates which were then developed with $CHCl_3$–EtOH (19:1). After spraying with *Cladosporium cucumerinum* spore suspension and incubating for 48 hr in a moist atmosphere, no inhibition zones were observed in the diseased wood extract, a minor zone occurred at R_f 0.33 in samples of both healthy and interface wood extracts, and a very large inhibitory zone occurred at R_f 0.57 in the interface sample alone.

Shavings of the pigmented interface wood (250 g) were extracted with EtOH (2 l.), evaporated and chromatographed on a column of Merck Kieselgel 40. 7-Hydroxycalamenene was eluted with $CHCl_3$–EtOH (49:1) and further purified by prep. TLC on Si gel (Merck) with the same solvent system. It was obtained as a gum (2.31 g, 0.92% of interface fr. wt), homogeneous on GC (R_t 4.1 min, 3% OV-225 on Gas Chrom Q, 174°, 30 ml N_2/min, FID), MS *m/z* 218 (M^+ 5%), 175 (100), 160 (8), 145 (7), 121 (4). $[\alpha]_D$ −33° ($CHCl_3$; *c* 2.0) (lit. −30° [3]). UV λ_{max} nm: 281 (lit. 280 nm [3]). ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.77 (3H, *d*, *J* = 7 Hz), 1.00 (3H, *d*, *J* = 7 Hz), 1.22 (3H, *d*, *J* = 7 Hz), 1.68 (5H, *m*), 2.19 (3H, *s*), 2.60 (2H, *m*), 4.68 (1H, *br s*), 6.53 (1H, *s*), 6.90 (1H,

s). The 3,5-dinitrobenzoate, prepared by refluxing with 3,5-dinitrobenzoyl chloride in pyridine, crystallized from hexane–EtOAc as yellow needles, mp 135–136° (lit. 136–137° [3]).

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HELIANTHOL A, A SESQUITERPENE ALCOHOL FROM *HELIANTHUS TUBEROSUS**

MITSUO MIYAZAWA and HIROMU KAMEOKA

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Kowakae, Higashiosaka-shi 577, Japan

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Key Word Index—*Helianthus tuberosus*; Compositae; essential oil; helianthol A; sesquiterpene alcohol.

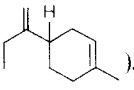
Abstract—A new sesquiterpene alcohol, helianthol A, was isolated from the aerial parts of *Helianthus tuberosus*. The structure of this compound has been established as (+)-2-methyl-6-[4-methyl-3′-cyclohexen-1′-(*R*)-yl]-3,6-heptadien-2-ol by chemical and spectroscopic methods.

INTRODUCTION

Helianthus tuberosus L. has been analysed chemically and the sesquiterpene heliangine was found [1–4]. We have now isolated a new sesquiterpene alcohol from the essential oil of the aerial parts.

RESULTS AND DISCUSSION

The essential oil was obtained by steam distillation of the aerial parts of *H. tuberosus* which were collected from the outskirts of Osaka, Japan. The compound (helianthol A) (**1**), 18.0% of the essential oil, was isolated by alumina column chromatography and by prep. GC, using Celite 545 as the stationary phase. Helianthol A, $[\alpha]_D^{20}$ +61.0° (EtOH; *c* 0.3), has the molecular formula $C_{15}H_{24}O$ (M^+, *m/z* 220, 0.2%), based on mass spectrometry and the following physical properties. IR ν_{max} cm^{-1}: 3360 1140 (tertiary hydroxyl), 3080, 1635, 890 (terminal olefin). ^1H NMR: δ ($CDCl_3$, TMS) 1.33 (6H, *s*, Me_2–C–OH), 1.65 (3H, *s*, Me–C=CH–), 2.02 (4H, *m*, $-CH_2-CH=$), 2.15 (1H, *m*,



), 2.75 (2H, *dd*, *J* = 15 Hz, $=CH-CH_2-C=$), 4.75 (2H, *m*, $CH_2=$), 5.38 (1H, *m*, –CH=C–), 5.64 (2H, *m*, –CH=CH–), 1.45 (1H, *s*, –OH, on deuteration shows no signal). MS: *m/z* 220 (M^+, 0.2%), 79 (100), 91(82), 93 (73), 119 (71), 105 (69), 202 (22), 187 (13). This compound has a similar skeleton to β-bisabolene the main component of this oil. Its structure as a related alcohol was confirmed by the following procedures. When the compound was heated with 10% aqueous oxalic acid for 4 hr, the dehydration product (**2**) was obtained. These results confirmed that the position of the hydroxy group was at C-2. In addition, the dehydration product **2**, was reduced with hydrogen on platinum oxide and was identified as a bisabolane, **3**. The ^{13}C NMR figure and the data on the ^{13}C-signals which were assigned by ^1H off-resonance are summarized in Table 1. The appearance of the carbon signal of the solvent ($CDCl_3$) at δ 78.30–75.76 (*t*) and another 15 signals are shown in Table 1. The signals of C-1 and C-8 were found at a high magnetic field at δ 29.87 and appeared equal in value to each other. Also observed was the appearance of a carbon (δ 70.72), on C-2, attached to a hydroxyl group in **1** which supported the partial structure

*Part X in the series "Chemical Constituents of Naturalized Plants of Japan".