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INSECT SEX PHEROMONE TYPE ALKENES FROM THE SEEDS OF *Quercus robur*

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A combination of vacuum liquid chromatography and preparative thin layer chromatography of the combined n-hexane and dichloromethane extracts of the seeds of Quercus robur *afforded two insect sex pheromone type alkenes, 5E-tetradecen-1-ol (**1**) and 6E-tetradecen-1-ol (**2**), none of which has ever been isolated from any plant sources. The structures of these alkenes were determined by spectroscopic techniques.*

Key words: *Quercus robur*, Fagaceae, pheromone analogue, 5*E*-tetradecen-1-ol, 6*E*-tetradecen-1-ol.

Quercus robur L. (family: Fagaceae), commonly known as "English oak, "pedunculate oak", or "European oak", is a a majestic British deciduous tree (30–40 m) with a wide spreading crown, a short sturdy trunk, and deeply fissured grey brown bark [1, 2]. It is found extensively in a number of other countries of Europe, temperate Asia, and northern Africa. English oak is used as a shade tree or a specimen tree in larger landscapes. The wood has been a valuable commodity for centuries, and during Britain's reign on the High Seas, many a sailing ship was made from the fine hard wood of English oak. The bark of *Q. robur* has astringent and emollient properties. It has traditionally been taken internally as an infusion as a remedy for haemorrhages, diarrhoea and intermittent fevers, and externally as an ointment to treat haemorrhoids. The acorn of this plant is also an astringent, and has been employed as an old traditional remedy for diarrhoea. Polyphenols [3–12], triterpenes [13], a-tocopherol [14], benzoquinones [15] and volatile compounds [16] have previously been reported from *Q. robur*. We now report on the isolation and structure elucidation of two insect sex pheromone like alkenes, 5*E*-tetradecen-1-ol (**1**) and 6*E*-tetradecen-1-ol (**2**), from the seeds of this plant.

A combination of vacuum liquid chromatography (VLC) and preparative TLC of the combined *n*-hexane and dichloromethane (DCM) extracts of the seeds of *Quercus robur* afforded, 5*E*-tetradecen-1-ol (**1**) and 6*E*-tetradecen-1-ol (**2**), the structures of which were elucidated by spectroscopic means.

The HR-EIMS spectra of both **1** and **2** revealed the molecular ions, respectively, at *m/z* 212.214 and 212.2140, calculated for 212.2140 for $C_{14}H_{28}O$. In the EIMS spectra, a fragment ion at *m/z* 194 [M-18] represented the ion originated from the loss of a water molecule from the compound and suggested that these compounds were alcohols. The IR spectra of **1** and **2** showed the absorption signals for the alcoholic hydroxyl group (3323 and 3320 cm^{-1}) and sp^2 hybridised carbons (3006 and 3004 cm^{-1}, and 1600–1400 cm^{-1}) in the molecules. The ^1H NMR spectrum of **1** exhibited a 3H triplet at δ 0.86 (J = 6.7) and a 2H triplet at δ 4.15 (J = 7.0), typical for a terminal methyl and oxymethylene groups in a long-chain fatty alcohol. The signals at δ 5.33 and 5.24, each integrated for single proton, could be assigned to two olefinic methines. The coupling constants J = 15.9 Hz confirmed their orientation as *trans*. In addition to these signals, there were signals (δ 1.28–2.75) for ten methylene groups. The ^{13}C NMR showed signals for all 14 carbons including a methyl (δ 14.1), two olefinic methines (δ 130.1 and 129.7), one oxymethylene (δ 62.1) and ten methylene carbons (δ 22.7– 34.2). The placement of the double bond between C-5 and C-6 was confirmed from ^1H–^{13}C HMBC correlations (Fig. 1). The oxymethylene protons (H-1, δ 4.15) showed ^2J and ^3J correlations, respectively, to C-2 (δ 31.9) and C-3 (29.7), the latter was also correlated (^3J) to H-5 (δ 5.24). The olefinic proton H-6 (δ 5.33) showed ^3J correlations to C-4 (δ 34.0) and C-8 (δ 29.7). Thus the placement of the double bond in between C-5 and C- 6 was confirmed. The experimental spectroscopic data of **1** were in good agreement with published data for 5*E*-tetradecen-1-ol [17].

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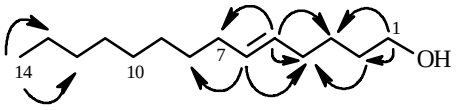

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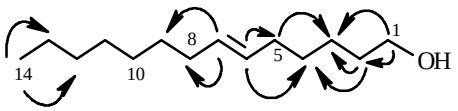

2

Fig. 1. Key ^{1}H–^{13}C HMBC ling-range correlations in compounds **1** and **2**.

The ^{1}H and ^{13}C NMR data of **2** were very similar to those of **1** which indicated that **2** was actually a positional isomer of **1**. The ^{1}H–^{13}C HMBC (Fig. 1) confirmed that the only difference between **1** and **2** was in the position of the double bond in between C-6 and C-7 in **2** (instead of between C-5 and C-6 in **1**) (Fig. 1). The experimental spectroscopic data of **2** were in good agreement with published data for 6*E*-tetradecen-1-ol [18–20].

This is the first report on the occurrence of insect sex pheromone like alkenes (**1** and **2**) in the seeds of *Q. robur*. To our knowledge, none of these compounds has ever been isolated from any plant sources. However, the acetylated derivative of **1**, 1-acetoxy-5-tetradecene, which is a sex pheromone for brown headed leafroller *Ctenopseustis obliquana*, was reported from a plant source, *Hibiscus abelmoschus* [17, 21]. The acetylated derivative of **2**, 1-acetoxy-6-tetradecene, is a component of sex pheromones of the apple ermine moth, *Yponomeuta malinellus*.

Quercus robur is considered to be an ideal host for about 38 different parasites and is prone to insect and fungal attacks which often lead to canker. It is a host plant for various insect species and pests, e.g. *Alebra albostriella, Cameraria hamadryadella, Cirrospilus diallus, Diadegma anurum, Pnigalio arraules, Scambus annulatus, Tischeria ekebladella,* etc. [16, 22–29]. Production of compounds **1** and **2** in *Q. robur* is possibly the result of plant-insect interaction. It has been observed that changes in plant chemistry, especially in relation to the production of various sex pheromone proxys, could sometimes be induced by phytophagous insects to provide cues for mate location [30]. Plant secondary metabolites are most often insect deterrent but stimulate phagostimulatory cells if they serve as host-indicating sign stimuli, or if they are sequestered for defence or used as pheromone precursors [31, 32] It has been shown that host plant selection relies on the balance of phagostimulatory and deterrent inputs with a prominent role of a host-related chemical. Compounds **1** and **2** are present in quite high amounts, respectively, 2.4×10^{-2} and 2.2×10^{-2}%, in *Q. robur*. Therefore, the occurrence of these insect hormones like alkenes in this plant might have some ecological implications.

EXPERIMENTAL

General Procedures. IR spectra (nujol) were obtained using an AVATAR 360 FT-IR spectrometer. NMR spectra were recorded in $CDCl_3$ on a Varian Unity INOVA 400 MHz NMR Spectrometer 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) using the residual solvent peaks as internal standard. EIMS and HREIMS analyses were performed on a Finnigan MAT95 XP spectrometer. VLC and prep TLC were carried out using, respectively, Merck Silica gel 60H and Merck Silica gel 60 G. HMBC spectra were optimised for a long range $J_{H\text{-}C}$ of 9 Hz.

Plant Material. The seeds of *Q. robur* L. (cat. no. 27187) were purchased from a commercial seed supplier, B & T World Seeds Sarl, Paguignan, 34210 Olonzac, France. A voucher specimen (PH700210) has been deposited in the herbarium of Plant and Soil Science Department, University of Aberdeen, Scotland (ABD).

Extraction, Isolation, and Structure Elucidation. Dried seeds (94.0 g) of *Q. robur* were ground using a coffee grinder and Soxhlet-extracted, successively, with *n*-hexane, DCM and MeOH (1.1 L each). The *n*-hexane and DCM extracts were combined and subjected to VLC eluting with solvent mixtures of increasing polarity, *n*-hexane, *n*-hexane-EtOAc, EtOAc, EtOAc–MeOH, and finally MeOH. Preparative TLC (mobile phase: 10% EtOAc in *n*-hexane) of the combined VLC fractions 5 & 6 (15% & 20% EtOAc in *n*-hexane) resulted in the isolation of two insect pheromone analogues, 5*E*-tetradecen-1-ol (**1**, 23.3 mg, R_f 0.71) [17] and 6*E*-tetradecen-1-ol (**2**, 21.1 mg, R_f 0.74) [18–20]. The structures of **1** and **2** were determined by a combination of IR, HR-EIMS, EIMS, and extensive 1D and 2D NMR analyses.

5*E*-Tetradecen-1-ol (1). IR ν_{max} (nujol) cm^{-1}: 3323, 3006, 2955, 2929, 2845, 1634, 1470, 1460, 1400, 1372, 1302, 1126, 1070, 870, 865, 790, 721; HR-EIMS *m/z* found 212.2141 calc. 212.2140 for $C_{14}H_{28}O$. EIMS *m/z* (rel int.): 212 $[M]^{+}$ (20), 194 $[M\text{-}18]^{+}$ (40), 166 (10), 152 (12), 138 (10), 124 (12), 112 (40), 110 (20), 96 (60), 82 (100), 66 (70), 56 (82), 54 (30), 41 (75); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.33 (1H, dt, H-5, J = 15.9, 6.8), 5.24 (1H, dt, H-6, J = 15.9, 6.8), 4.15 (2H, br t, J = 7.0, H-1), 2.75 (2H, m, H-2), 2.30 (2H, m, H-4), 1.98 (2H, m, H-7), 1.55 (2H, m, H-3), 1.28 (12H, m, H-8, H-9, H-10,

H-11, H-12, H-13), 0.86 (3H, t, J = 6.7, 14-Me); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 130.1 (C-5), 129.7 (C-6), 62.1 (C-1), 34.2 (C-7), 34.0 (C-4), 31.9 (C-2), 29.7 (C-3, C-8, C-9, C-10), 29.4 (C-11), 29.6 (C-12), 22.7 (C-13), 14.1 (C-14).

6*E*-Tetradecen-1-ol (2). IR ν_{max} (nujol) cm^{-1}: 3320, 3004, 2960, 2924, 2846, 1630, 1468, 1456, 1402, 1370, 1308, 1129, 1072, 872, 864, 796, 720; HR-EIMS *m/z* found 212.2140 calc. 212.2140 for $C_{14}H_{28}O$. EIMS *m/z* (rel int.): 212 $[M]^+$ (20), 194 $[M-18]^+$ (40), 166 (8), 152 (10), 138 (20), 124 (15), 112 (10), 110 (8), 98 (100), 96 (80), 66 (70), 56 (82), 54 (30), 41 (70); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.36 (1H, dt, H-6, J = 15.9, 6.8), 5.29 (1H, dt, H-7, J = 15.9, 6.8), 4.14 (2H, br t, J = 7.0, H-1), 2.30 (2H, m, H-2), 1.98 (2H, m, H-4), 1.59 (2H, m, H-7), 1.28 (14H, m, H-3, H-8, H-9, H-10, H-11, H-12, H-13), 0.84 (3H, t, J = 6.8, 14-Me); ^{13}C NMR (100 MHz, $CDCl_3$,δ, ppm): 130.1 (C-6), 129.9 (C-7), 62.0 (C-1), 34.1 (C-8), 33.9 (C-5), 31.9 (C-2), 29.7 (C-3, C-4, C-9, C-10), 29.4 (C-11), 29.3 (C-12), 22.9 (C-13), 14.2 (C-14).

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