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The proposed structures of phenolic compounds isolated from *Piper betle* L. differ from those of the compounds obtained by total synthesis

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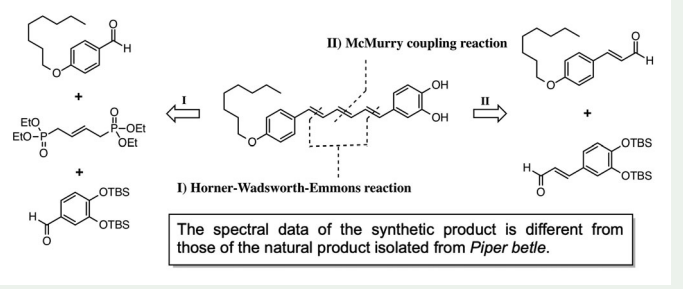
ABSTRACT

We describe the syntheses of phenolic compounds, 4-[(1*E*, 3*E*, 5*E*)−6-(4-octyloxyphenyl)hexa-1,3,5-trien-1-yl]benzene-1,2-diol (**1**) and 3-(*n*-dodecyloxy) phenol (**2**), isolated from *Piper betle*. The triene moiety of 4-[(1*E*, 3*E*, 5*E*)−6-(4-octyloxyphenyl)hexa-1,3,5-trien-1-yl]benzene-1,2-diol was formed *via* two different methods, the Horner–Wadsworth–Emmons reaction and the McMurry coupling reaction. The spectral data of synthesized compounds show differences with those of reported as the naturally occurring compounds.



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Piper betle; Horner–Wadsworth–Emmons reaction; McMurry coupling; total synthesis

1. Introduction

Phenolic compounds (**1** and **2**, Figure 1) have been isolated from *Piper betle* L. by Atiya et al. (2018 and 2020); these compounds have been reported to be secondary metabolites that exhibit excellent DPPH free radical scavenging activities and moderate cytotoxic activities (Atiya et al., 2018 and 2020). The structure of **1** was proposed as 4-[(1*E*, 3*E*, 5*E*)−6-(4-octyloxyphenyl)hexa-1,3,5-trien-1-yl]benzene-1,2-diol on the basis of spectroscopic analyses. We were interested in the unique structure of compound **1**, which

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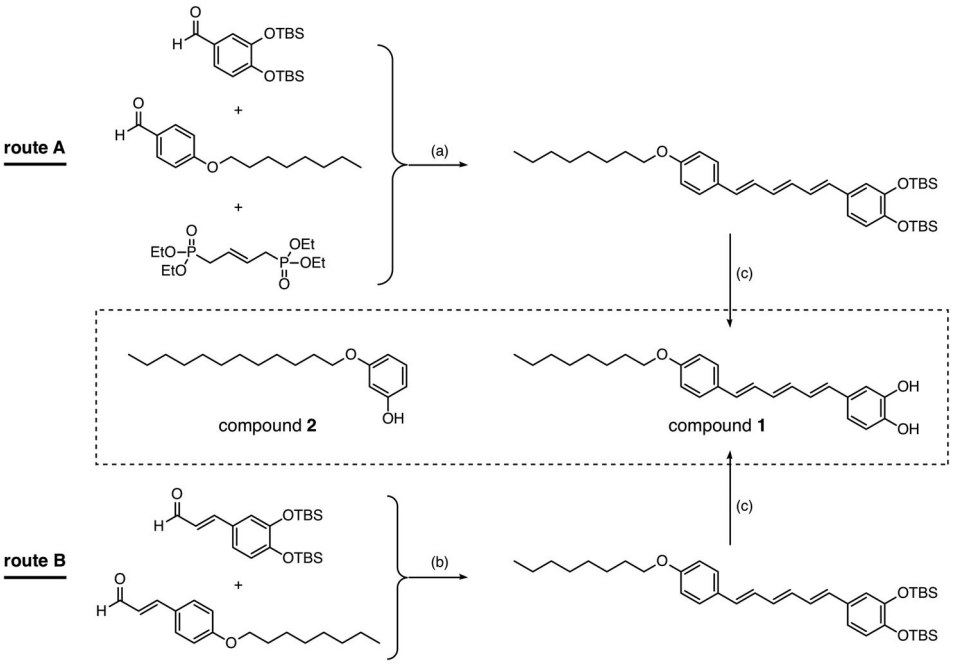

Figure 1. Proposed structures for phenolic compounds **1** and **2** isolated from *Piper betle* and those obtained by synthetic routes to produce triene **1**. Reagents and conditions: (a) *t*-BuOK, THF, room temperature, 1 h; (b) zinc powder, $TiCl_4$, pyridine, THF, 60 °C (MW), 5 min; (c) TBAF, THF, room temperature, 4 h, 41% in two steps *via* route A, 29% in two steps *via* route B.

has a very rare structure as the natural products containing two C6 units that are connected by a triene. As such, confirmation of the structure of the natural product is of particular importance from the viewpoint of natural products chemistry. On the other hand, compound **2** is a very simple and common compound in contrast to **1**, but it is interesting to note that antioxidant activity comparable to ascorbic acid (vitamin C) has been reported.

Thus, we herein report the syntheses of compounds **1** and **2**, however, the NMR data of synthesised compounds were not consistent with the reported natural products data. These results thereby indicate that the structures of the phenolic compounds **1** and **2** isolated from *P. betle* require reinvestigation.

2. Results and discussion

The first synthetic route (route A) for the synthesis of 4-[(1*E*, 3*E*, 5*E*)−6-(4-octyloxyphenyl)hexa-1,3,5-trien-1-yl]benzene-1,2-diol (**1**) is shown in Figure 1. In this synthetic route, the formation of the triene moiety was performed *via* the Horner–Wadsworth–Emmons (HWE) reaction, which is based on the method used by Ha et al. (2013) for syntheses of 1,6-bis(substituted phenyl)hexa-1,3,5-trienes. The starting aldehydes, 4-(octyloxy)benzaldehyde and 3,4-bis[(1,1-dimethylethyl)dimethylsilyloxy]benzaldehyde were prepared in a conventional manner from 4-hydroxybenzaldehyde and 3,4-dihydroxybenzaldehyde, respectively, and diphosphonate was prepared as described by Ha et al. (2013). The

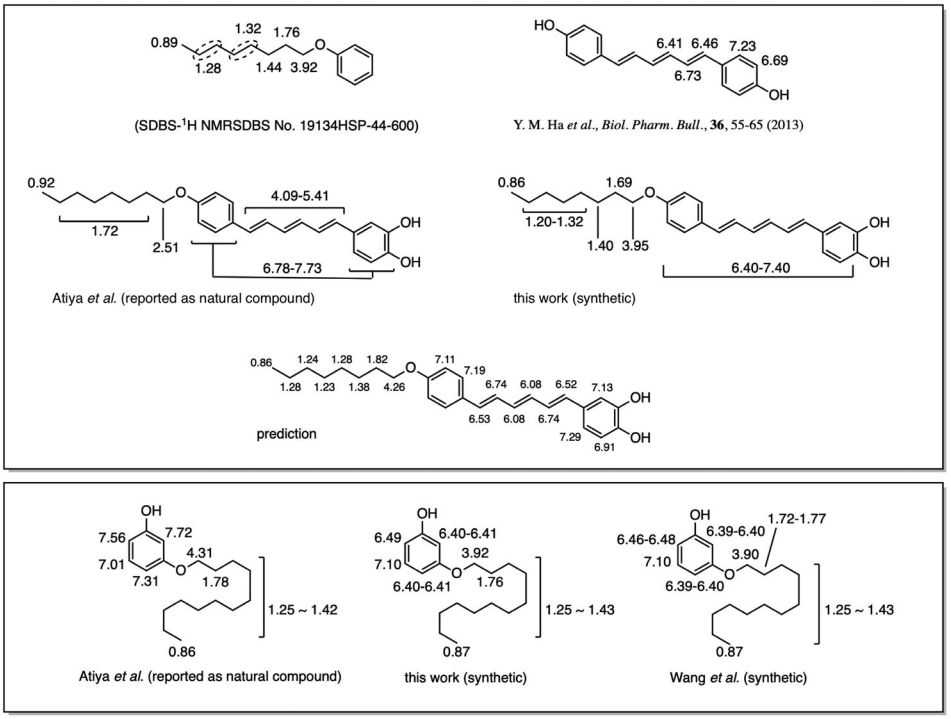

Figure 2. Comparison of the ^{1}H NMR spectral data for synthesised, reported as natural compounds and reported similar compounds.

HWE olefination reaction of two aldehydes and diphosphonate produced an inseparable triene mixture. The resulting mixture was treated with tetra-*n*-butylammonium fluoride (TBAF) to produce highly polar compounds including the desired **1**. Purification by silica gel column chromatography yielded pure compound, which is considered to be triene **1**. In the ^{1}H NMR spectra of synthetic **1**, the presence of a broad signal of phenolic hydroxy protons at 8.84-9.26 ppm and signals derived from the alkyl group (0.86, 1.20–1.32, 1.40 and 1.69 ppm) indicate the success of deprotection following the coupling reaction. In addition, the detection of the pseudomolecular ion at *m/z* 391.2263 $[M–H]^-$ is consistent with the molecular formula of $C_{26}H_{32}O_3$, which confirmed the total synthesis of compound with the desired structure.

The reported ^{1}H NMR data for natural **1** is shown in Figure 2. The comparison of the data for synthetic **1** with those reported for natural **1** reveals a distinct difference. In addition, synthetic **1** is poorly soluble in methanol and chloroform; therefore, DMSO-d_6 was used to obtain NMR spectra, whereas Atiya et al. (2020) measured the spectra of natural **1** in methanol-d_4 and chloroform-*d*. This suggests that the physical properties are different, which implies that either the structure of the natural product determined by Atiya et al. (2020) or the synthesis performed by us is wrong. Therefore, we decided to synthesise compound **1** with reported structure by another route and compare the data with that reported for natural product **1**.

The second synthetic route (route B) is shown in Figure 1. In this synthetic route, the construction of the triene moiety was performed by the microwave-assisted McMurry coupling reaction, which is based on the method used by Ramama et al.

(2004). Specifically, two aldehydes (i.e., coupling substrates) were prepared in a usual manner (Demin et al. 2004; Kumar et al. 2006). The McMurry coupling of two α, β-unsaturated aldehydes produced inseparable crude trienes that contained the desired triene as major product (ca. 62% by HPLC analysis) and minor dimeric trienes. The McMurry reaction product was subjected to the next deprotection without purification, and the obtained compound was the same triene made by the previous another method.

The same compound was obtained by a different synthetic route, which suggests a possibility of an error in the structural analysis of the natural product. Therefore, it was decided to solve this problem by analysing the spectra of similar compounds, as shown in Figure 2. First, the chemical shift of methylene protons adjacent to oxygen (–CH_2–O–) was examined. The synthesised compound indicated a peak at 3.95 ppm; however, the natural compound indicated a peak at 2.51 ppm. The chemical shift of the –CH_2–O– proton in (*n*-octyloxy)benzene was observed at 3.92 ppm (AIST 2019), which strongly suggests that assignments for the natural product may be wrong. Next, triene protons were checked. The shift for these protons were observed with those of benzene at 6.40–7.40 ppm as inseparable signals for synthetic compound **1** and at 4.09–5.41 ppm for natural compounds. For example, the chemical shifts of triene protons of 4,4′-[(1*E*, 3*E*, 5*E*)-hexa-1,3,5-triene-1,6-diyl]diphenol were observed at 6.41, 6.46 and 6.73 ppm (Ha et al. 2013). This result indicated a mistake in the structure determination of the natural compound. In addition, the results of spectral calculations from online NMR prediction services (nmrdb.org 2020) reinforce our conclusions.

Next, we synthesised 3-(*n*-dodecyloxy)phenol by microwave-assisted half alkylation of the hydroxy group of catechol (Paul and Gupta 2004). The ^{1}H NMR spectral data of the obtained compound and that of the natural compound are shown in Figure 2. The reported NMR data for natural 3-(*n*-dodecyloxy)phenol (**2**) and synthesised **2** was compared. However, differences exist between the data even though they were obtained using the same solvent. Therefore, in comparison with the spectral data of this previously synthesised compound (Wang et al. 2006), the data in this study are in complete agreement, as shown in Figure 2. Therefore, it is clear that there was an error in the structure determination by Atiya; however, no revised structure can be proposed. The synthesised 3-(*n*-dodecyloxy)phenol exhibited a moderate radical scavenging activity (data not shown), which was expected because compound **2** is a mono-ether of resorcinol.

At this stage, it is unclear whether the correct structure was determined for naturally occurring **1** and **2**. However, given the synthetic route that was used and the NMR and HRMS data, the determined structures for synthesised **1** and **2** are likely correct. The differences in the NMR data between synthesised and naturally occurring **1** and **2** suggest the possibility of an error in the interpretation of the data used for the structural determination of naturally occurring **1** and **2**.

3. Experimental section

3.1. General

All solvents were of a reagent grade. All commercial reagents were of the highest purity available and were purchased from FUJIFILM Wako pure chemical (Osaka, Japan),

Nacalai tesque (Kyoto, Japan), Tokyo chemical industry (Tokyo, Japan) and Kanto Chemical (Tokyo, Japan). Infrared (IR) spectra were obtained using a Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a diamond horizontal attenuated total reflectance (ATR) accessory; the final spectra were the result of co-addition of 16 interferograms. ^{1}H and ^{13}C NMR were obtained with an Agilent 400-MR (Agilent, Santa Clara CA, USA) spectrometer. HRMS was carried out in an electrospray ionisation mode using an Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Analytical TLC was performed on Merck Silica gel 60 F_{254}. Crude products were purified by column chromatography on a Silica Gel 60 N [Kanto chemical, particle size (spherical, neutral) of 100–210 μm].

3.1.1. Preparation of 4-[(1E, 3E, 5E)-6-(4-octyloxyphenyl)hexa-1,3,5-trien-1-yl] benzene-1,2-diol (1) via the horner–wadsworth–emmons (HWE) reaction

A solution of (*E*)-tetraethyl but-2-ene-1,4-diyldiphosphonate (400 mg, 1.2 mmol) in tetrahydrofuran (THF) and a solution of 4-(octyloxy)benzaldehyde (240 mg, 1.0 mmol) and 3,4-bis[(1,1-dimethylethyl)dimethylsilyloxy]benzaldehyde (360 mg, 1.0 mmol) in THF were added to a stirred suspension of potassium *tert*-butoxide (340 mg, 3.0 mmol) in THF at ice-cooling temperature; then, the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was poured into a dilute HCl solution (ca. 3%). The mixture was extracted with $CHCl_3$ three times, and the combined organic layer was washed with brine and dried with $CaCl_2$. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (benzene-AcOEt = 20/1) to yield crude trienes. A 1 M TBAF solution of THF (2 mL) at ice-cooling temperature was added to a solution of crude trienes in THF, and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was poured into a dilute HCl solution (ca. 3%). The mixture was extracted with $CHCl_3$ three times, and the combined organic layer was washed with brine and dried with $CaCl_2$. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (benzene-AcOEt = 8/1) to yield 4-[(1*E*, 3*E*, 5*E*)−6-(4-octyloxyphenyl)hexa-1,3,5-trien-1-yl]benzene-1,2-diol (**1**, 160 mg, 0.4 mmol, 41% in two steps). **1**: light brown amorphous powder. IR (diamond-ATR)ν_{max} 3381, 2921, 1602, 1508, 1249, 984, 860 and 798 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, $J = 8.6$ Hz), 1.20–1.45 (10H, m), 1.69 (2H, m), 3.95 (2H, t, $J = 6.3$ Hz), 6.40–6.58 (3H, m), 6.63–6.93 (8H, m), 7.40 (2H, d, $J = 8.3$ Hz), 8.84–9.26 (2H, br). ^{13}C NMR (DMSO-d_6) δ 14.0, 22.1, 25.5, 28.7, 28.8, 31.3, 34.4, 67.5, 113.1, 114.7, 115.8, 118.5, 124.9, 126.2, 127.3, 127.5, 128.1, 128.9, 129.8, 131.2, 132.3, 132.4, 133.1, 145.4, 145.6, 158.3. HRMS *m/z* 391.2263 $[M–H]^-$ (calcd. for $C_{26}H_{31}O_3$ 391.2268).

3.1.2. Preparation of 4-[(1E, 3E, 5E)-6-(4-octyloxyphenyl)hexa-1,3,5-trien-1-yl] benzene-1,2-diol (1) via the McMurry coupling reaction

Under an N_2 atmosphere, the suspension of zinc powder (130 mg, 2.0 mmol) in THF was cooled to ice-cooling temperature, and $TiCl_4$ (0.2 mL, 2.0 mmol) was slowly added dropwise. The suspension mixture was warmed to room temperature, and two drops of pyridine were added and stirred for 30 min. The solution of two aldehydes, 3,4-bis[(1,1-dimethylethyl)dimethylsilyloxy]cinnamaldehyde (390 mg, 1.0 mmol) and 4-(octyloxy)cinnamaldehyde (260 mg, 1.0 mmol), in THF was added dropwise. After the

addition, the reaction mixture was heated at reflux for 5 min using the microwave reactor (μReactor Ex, Shikoku Instrumentation Co., Ltd, Kagawa, Japan). Then, the reaction mixture was poured into a 10% K_2CO_3 aqueous solution and extracted with CH_2Cl_2. The organic layer was combined and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (benzene–AcOEt = 20/1) to yield crude trienes. A 1 M TBAF solution of THF (2 mL) at ice-cooling temperature was added to the solution of the residue including trienes, and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was poured into a dilute HCl solution (ca. 3%). It was extracted with $CHCl_3$ three times, and the combined organic layer was washed with brine and dried with $CaCl_2$. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (benzene–AcOEt = 8/1) and preparative thin-layer chromatography (benzene–AcOEt = 10/1) to yield 4-[(1*E*, 3*E*, 5*E*)−6-(4-octyloxyphenyl)hexa-1,3,5-trien-1-yl]benzene-1,2-diol (**1**, 113 mg, 0.29 mmol, 29% in two steps). All spectral data were in complete agreement with those of **1** synthesised *via* the HWE reaction.

3.1.3. Preparation of 3-(n-dodecyloxy)phenol (2)

A mixture of resorcinol (550 mg, 5.0 mmol), Zinc powder (65 mg), 1-bromododecane (1250 mg, 5.0 mmol) and *N,N*-dimethylacetamide (8 mL) in microwave vial was irradiated at 160 °C for 45 min. The reaction mixture was filtrated. After the addition of diluted HCl, the aqueous solution was extracted with AcOEt five times, and the combined organic layer was washed with brine and dried with $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$-MeOH = 30/1) to yield 3-(*n*-dodecyloxy)phenol (**2**, 500 mg, 1.8 mmol, 36%). IR (diamond-ATR)ν_{max} 3400, 2919, 1614, 1586, 1492, 1279, 1148 and 1029 cm^{-1}; The ^{1}H-NMR spectral data (see Figure 2) was in complete agreement with previously synthesised **2** (Wang et al. 2006). negative-ion HRESIMS m/z 277.2173 $[M-H]^-$ (Calcd for $C_{18}H_{29}O_2$, 277.2162).

4. Conclusion

In this paper, we described the syntheses of 4-[(1*E*, 3*E*, 5*E*)−6-(4-octyloxyphenyl)hexa-1,3,5-trien-1-yl]benzene-1,2-diol and 3-(*n*-dodecyloxy)phenol (i.e., the proposed structures for the naturally occurring DPPH free radical scavengers isolated from *Piper betle*). However, the spectral data of the reported and the synthesised compounds considerably differed. These results suggest that the structures of DPPH free radical scavengers isolated from *Piper betle* require reinvestigation.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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