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Full Length Research Paper

Secondary Metabolites from *Cinnamomum cebuense*

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Cinnamomum cebuense **Kosterm (Lauraceae) is an endemic and endangered Philippine tree yielded used by local residents as remedy for stomach ache. Chromatographic separation of the dicloromethane extract of the bark of *C. cebuense* gave rise to safrole and eugenol which were identified by NMR spectroscopy. Safrole is a known hepatocarcinogen, while eugenol is reported to be cytotoxic. The dichloromethane extracts of the air-dried leaves and roots of *C. cebuense* contain polyprenol and trilinolein, respectively. Polyprenols are reported to exhibit hepatoprotective effects, while trilinolein is reported to exhibit myocardial protective effects and it inhibits the endothelin-1-induced hypertension.**

Key words: *Cinnamomum cebuense,* Lauraceae, eugenol, safrole, polyprenol, trilinolein.

INTRODUCTION

Cinnamomum cebuense Kosterm. (Lauraceae), commonly known as kaningag or kalingag, is an endemic and endangered Philippine tree, found only in Cebu Island, Philippines. The sample which was collected at the Department of Environment and Natural Resources Protected Site located in Sitio Cantipla, Barangay Tabunan, Cebu City was described by Kostermanns in 1986. The bark of the tree is used by local residents as remedy for stomach ache, whereby the bark is either chewed directly or boiled with a glass of water before intake (Global Trees Campaign, 2011).

We earlier reported the isolation of a new monoterpene natural product and a new sesquiterpene, along with the known compounds, 4-hydroxy-3-methoxycinnamaldehyde, 4-allyl-2-methoxyphenol or eugenol, α-terpineol and humulene from the bark of *Cinnamomum cebuense*, while the leaves contain humulene, β-caryophyllene, squalene, and a mixture of α-amyrin, β-amyrin and bauerenol (Ragasa et al.,2011 in-press). We report herein the isolation of the major constituents of the bark, safrole (1) and eugenol (2); leaves, polyprenol (3); and roots, trilinolein (4) (Figure 1) from another collection of *C. cebuense.* This is the first report on the isolation of 1, 3 and 4 from the tree.

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MATERIALS AND METHODS

General experimental procedures

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh), while the TLC was performed with plastic backed plates coated with silica gel F_{254}. The plates were visualized with vanillin-H_2SO_4 and warming.

Plant material

The bark, roots and leaves of *C. cebuense* were collected from the Department of Environment and Natural Resources protected site located at Sitio Cantipla, Brgy. Tabunan, Cebu City, Philippines in May 2011 by virtue of Wildlife Gratuitous Permit No. 2011-01.

Extraction and isolation of constituents from the bark of *C. cebuense*

The air-dried bark (230 g) of *C. cebuense* was soaked in

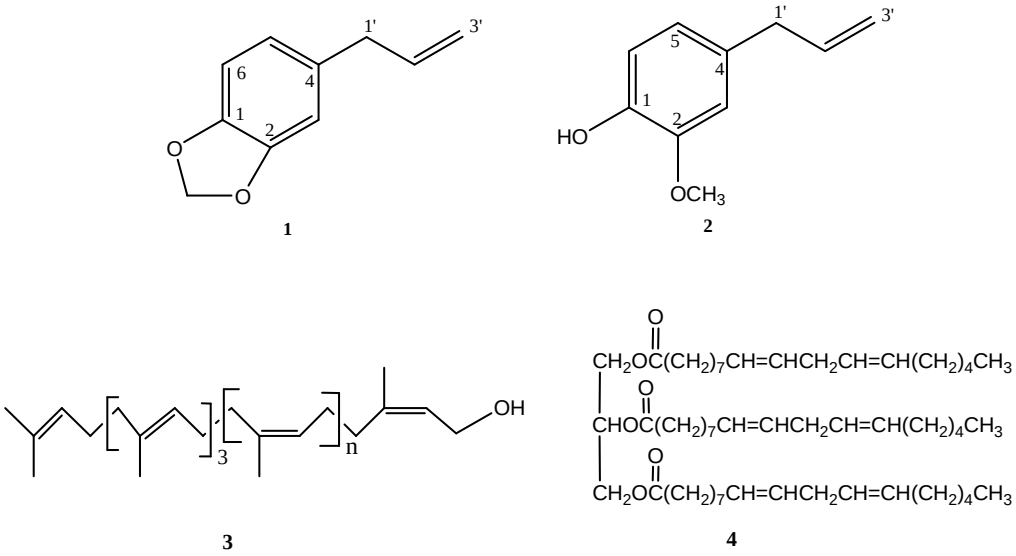

Figure 1. Chemical constituents of *Cinnamomum cebuense* bark, Safrole (1) and Eugenol (2); leaves, Polyprenol (3); and roots, Trilinolein (4).

dichloromethane for three days, and then filtered. The filtrate was concentrated under vacuum to produce the crude extract (4.42 g). The crude extract was fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% by volume increment) as eluents. The 10% acetone in dichloromethane fraction was rechromatographed in 10% ethyl acetate in petroleum ether. The less polar fractions were rechromatographed (3x) in 5% ethyl acetate in petroleum ether to yield 1, while the more polar fractions were rechromatographed (4x) in 7.5% ethyl acetate in petroleum ether to yield 2.

Extraction and isolation of constituents from the leaves of *C. cebuense*

The air-dried leaves (35.8 g) of *C. cebuense* was soaked in dichloromethane for three days, and then filtered. The filtrate was concentrated under vacuum to produce the crude extract (1.80 g). The crude extract was fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% by volume increment) as eluents. The 10 - 20% acetone in dichloromethane fractions were combined and rechromatographed (4x) in 10% ethyl acetate in petroleum ether to yield 3.

Extraction and isolation of constituents from the roots of *C. cebuense*

The air-dried roots (61.8 g) of *C. cebuense* was soaked in dichloromethane for three days, and then filtered. The filtrate was concentrated under vacuum to produce the crude extract (2.76 g). The crude extract was fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% by volume increment) as eluents. The dichloromethane and 10% acetone in dichloromethane fractions were combined and rechromatographed (5x) in 5% ethyl acetate in petroleum ether to yield 4. 5-(2-Propenyl)-1,3-benzodioxole or Safrole (1): colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.66 (H-3, m), 6.71 (d, *J* = 7.8 Hz, H-6), 6.61 (m, H-5), 3.28 (H_2-1′, d, *J* = 6.6 Hz), 5.88 (H-2′, m), 5.02-5.07 (H_2-3′, m), 5.83 (2H, s, OCH_2O); ^{13}C NMR (150 MHz, $CDCl_3$): δ ^{13}C NMR (150 MHz, $CDCl_3$): δ 145.80 (C-1), 147.61 (C-2), 108.15 (C-3), 133.85 (C-4), 121.29 (C-5), 108.15 (C-6), 39.90 (C-1′), 137.59 (C-2′), 115.67 (C-3′), 100.78 (OCH_2O).

4-Allyl-2-methoxyphenol or Eugenol (**2**): yellowish oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.83 (H-6, d, *J* = 8.4 Hz), 6.66-6.68 (2H, m, H-3, H-5), 3.30 (H_2-1′, d, *J* = 6.8 Hz), 5.93 (H-2′, m), 5.02-5.07 (H_2-3′, m), 5.47 (s, 1-OH), 3.86 (2-OCH_3, s); ^{13}C NMR (150 MHz, $CDCl_3$): δ 143.87 (C-1), 146.40 (C-2), 111.06 (C-3), 131.91 (C-4), 121.16 (C-5), 114.20 (C-6), 39.88 (C-1′), 137.80 (C-2′), 115.51 (C-3′), 55.84 (2-OCH_3).

Polyprenol (3): ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.07 (2H, CH_2OH), 5.43 (1H, =CH), 5.05-5.12 (11H, =CH), 1.95-2.09 (40H, allylic CH_2), 1.75 (3H, allylic CH_3), 1.66 (21H, allylic CH_3), 1.59 (12H, allylic CH_3); ^{13}C NMR: δ 59.01 (CH_2OH), 139.90, 136.08, 135.37, 135.28, 135.23, 135.20, 134.97, 134.89, 131.25, 125.01, 124.98, 124.93, 124.87 124.51, 124.44, 124.39, 124.25, 124.22, 124.12, 39.72, 32.22, 32.19, 32.17, 31.98, 26.76, 26.67, 26.63, 26.39, 26.35, 26.29, 25.69, 23.45, 23.42, 23.36, 17.67, 16.00, 15.99.

Trilinolein (4): ^{13}C NMR (150 MHz, $CDCl_3$): δ 62.10 (glyceryl CH_2), 68.88 (glyceryl CH), 173.26 (C=O α), 172.84 (C=O β), 34.05 (C-2α), 34.19 (C-2β), 24.83 (C-3α), 24.86 (C-3β), 29.08 (C-4α), 29.05 (C-4β), 29.20 (C-5α), 29.27 (C-5β), 29.11 (C-6α), 29.18 (C-6β), 29.62 (C-7α), 29.66 (C-7β), 29.19 (both C-8), 130.01 (C-9α), 129.98 (C-9β), 128.06 (C-10α), 128.08 (C-10β), 25.63 (both C-11), 127.90 (both C-12), 130.22 (both C-13), 27.19 (both C-14), 29.36 (both C-15), 31.52 (both C-16), 22.57 (both C-17), 14.07 (both C-18).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the air-dried bark of *C. cebuense* yielded 1 and 2. The structures of 1 and 2 (Figure 1) were identified by NMR

spectroscopy and confirmed by comparison of their ^{1}H and ^{13}C NMR data with those reported in the literature for safrole (Lee et al., 2009) and eugenol (Chen et al., 2010), respectively. 5-(2-Propenyl)-1,3-benzodioxole or safrole (1) was reported as a weak hepatocarcinogen and its carcinogenic effect has been linked to the formation of stable safrole-DNA adducts. The study demonstrated that safrole treatment induced oxidative damage on rat hepatic tissue and glutathione played an important protective role. This oxidative damage may be involved in the hepatocarcinogenic effect of safrole (Liu et al., 1999). An earlier study reported the incidence of hepatocellular carcinomas in rats fed with safrole which was markedly increased by simultaneous administration of phenobarbital (Wislocki et al., 1977). Safrole is an important precursor in the synthesis of methylenedioxymethamphetamine (MDMA) or ecstasy, a drug used in psychiatric counseling (Swist et al., 2005). Safrole was also reported to possess fumigant toxicity and contact toxicity to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* Herbst adults (Huang et al., 1999).

A number of *Cinnamomum* species have been reported to contain safrole. The essential oil of *C. mollissimomum* is a natural source of safrole (Jantan and Goh, 1990). The other *Cinnamomum* species that have been reported to contain safrole are *C. impressicostatum* and *C. pubescens* (Ali et al., 2010), *C. cassia* (Lv et al., 2010), *C. rhyncophyllum* (Jantan et al., 2004), and *C. camphora* (Stubbs et al., 2004). However, a recent study reported that a traditional method of *C. carolinense* tea preparation, which boils the bark shavings, degrades and eliminates the safrole (Reynertson et al., 2005).

4-Allyl-2-methoxyphenol or Eugenol (2) was reported to be cytotoxic against HL-60 leukemia cells with a 50% cytotoxic concentration (CC50) of 0.38 mM (Hirata et al., 2005). In another study, eugenol was found to induce a reactive oxygen species-mediated apoptosis in HL-60 human promyelocytic leukemia cells (Yoo et al., 2005). It also demonstrated a cytotoxic effect on the human osteoblastic cell line U2OS in a dose dependent manner with an IC_{50} value of 0.75 mmol/L (Ho et al., 2006). The cytotoxicity of eugenol in human HFF fibrobalsts and human HepG2 hepatoma cells was increased in the presence of hepatic S-9 microsomal fraction from Aroclor-induced rats or hamsters (Babich et al., 1993). A previous study demonstrated that eugenol is actively metabolized in hepatocytes and suggested that its cytotoxic effects are due to the formation of a reactive intermediate, possibly a quinine methide (Thompson et al., 1991). Another study reported that Eugenol at a dose of 100 mg/kg i.p. was able to inhibit the growth of Ehrlich ascites by 28.88%. In solid carcinoma, it showed 24.35% tumor growth inhibition (Jaganathan et al., 2010). Recently, a study demonstrated the molecular mechanism of eugenol-induced apoptosis in human colon cancer cells (Jaganathan et al., 2011). It significantly reduced the incidence of MNNG-induced gastric tumors by suppressing NF-κB activation and modulating the suppression of NF-κB target genes that regulate cell proliferation and cell survival (Manikandan et al., 2011). In addition to the cytotoxic properties of eugenol, it also possesses significant antioxidant, anti-inflammatory, analgesic, local anesthetic and cardiovascular activities (Pramod et al., 2010). Eugenol significantly inhibited carrageenan-induced edema in rats at 200 mg/kg. At doses of 50, 75 and 100 mg/kg, it exhibited significant antinociceptive effect in the acetic acid-induced abdominal writhing (Daniel et al., 2009).

Silica gel chromatography of the dichloromethane extract of the air-dried leaves of *C. cebuense* was used to yield 3, while the roots yielded 4. The structure of 3 was identified by NMR spectroscopy and confirmed by comparison of its ^{1}H and ^{13}C NMR data with those reported in the literature for Polyprenol (Rideout et al., 2003). The structure of 4 was identified by NMR spectroscopy and confirmed by comparison of its ^{13}C NMR data with those reported in the literature for trilinolein (Alemany, 2002).

The hepatoprotective effects of Polyprenols from *Ginkgo biloba* against carbon tetrachloride induced hepatic damage in Sprague-Dawley rats were comparable and not significantly different from those of the standard drug Essentiale. This indicates that Polyprenols are potentially promising additive in drugs for liver diseases (Yang et al., 2011). Trilinolein is a tyrosinase inhibitor (Jeon et al., 2006) and an antibacterial/antifungal compound (Bajpai et al., 2010). Trilinolein exhibits myocardial protective effects via its antioxidant ability (Liu et al., 2004); also it inhibits the endothelin-1-induced hypertension (Chen et al., 2005).

Conclusions

The dichloromethane extract of the air-dried bark of *Cinnamomum cebuense* produced eugenol and safrole as the major constituents. Eugenol is a known cytotoxic compound, while safrole is a known hepatocarcinogen. Some *Cinnamomum* species are reported to contain safrole. However, a recent study reported that a traditional method of *C. carolinense* tea preparation, made by boiling the bark shavings, degrades and eliminates the safrole. The major constituents of the dichloromethane extracts of the leaves and roots of *C. cebuense* are Polyprenols and Trilinolein, respectively. Polyprenols have hepatoprotective effects, while trilinolein exhibits myocardial protective effects and it inhibits the endothelin-1-induced hypertension.

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