

Terpenoids and Sterols from the Endemic and Endangered Philippine Trees, *Ficus pseudopalma* and *Ficus ulmifolia*

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The dichloromethane extract of the air-dried leaves of the endemic and endangered Philippine trees, *Ficus pseudopalma* and *Ficus ulmifolia* afforded squalene, polyprenol, β -amyrin fatty acid ester, α -amyrin acetate and β -amyrin acetate. *F. pseudopalma* also yielded lupeol fatty acid ester, lupenone, oleanone, and ursenone, while *F. ulmifolia* also afforded lutein, lupeol acetate, β -carotene, phytol, α -amyrin fatty acid ester, sitosterol, and stigmasterol. Their structures were identified by NMR spectroscopy.

Key Words: Carotenoids, *Ficus pseudopalma*, *Ficus ulmifolia*, Moreaceae, phytol, polyprenol, sterols, triterpenes

INTRODUCTION

The diversity of Philippine plants is exceptional, however, many have yet to be studied for their chemical constituents. *Ficus pseudopalma* and *Ficus ulmifolia* are endemic and endangered Philippine trees with no reported chemical studies. Thus, this research was conducted to isolate and identify the dichloromethane soluble secondary metabolites in both trees. *F. pseudopalma* is a medicinal and ornamental tree found throughout the Philippines. A decoction of the leaves is used for the treatment of kidney stones and diabetes, while the young shoots are eaten as vegetables (Stuart 2008). On the other hand, *F. ulmifolia* has no reported medicinal properties. The hard and rough leaves are used to clean cooking utensils and to scour wood. The fruits are edible, but has little flavor (Jansen et al. 1991).

We report here the isolation and identification of the dichloromethane soluble constituents of *Ficus pseudopalma* and *Ficus ulmifolia*. *F. pseudopalma* afforded the triterpenes: ursenone (1c), β -amyrin fatty acid

ester (2a), α -amyrin acetate (1b), β -amyrin acetate (2b), oleanone (2c), lupeol fatty acid ester (3a), lupenone (3c), and squalene (4); and polyprenol (5), while *F. ulmifolia* yielded the triterpenes: α -amyrin fatty acid ester (1a), α -amyrin acetate (1b), β -amyrin fatty acid ester (2a), β -amyrin acetate (2b), lupeol acetate (3b), and squalene (4); polyprenol (5), phytol (6), β -carotene (7), lutein (8), stigmasterol (9), and sitosterol (10) (Figure 1). This is the first report on the isolation of the aforementioned compounds from *Ficus pseudopalma* and *Ficus ulmifolia*.

MATERIALS AND METHODS

General Experimental Procedures: NMR spectra were recorded on a Varian Unity Inova spectrometer in CDCl₃ at 500 MHz for ¹H NMR and 125 MHz for ¹³C 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 F₂₅₄. The plates were visualized with vanillin-H₂SO₄ and warming.

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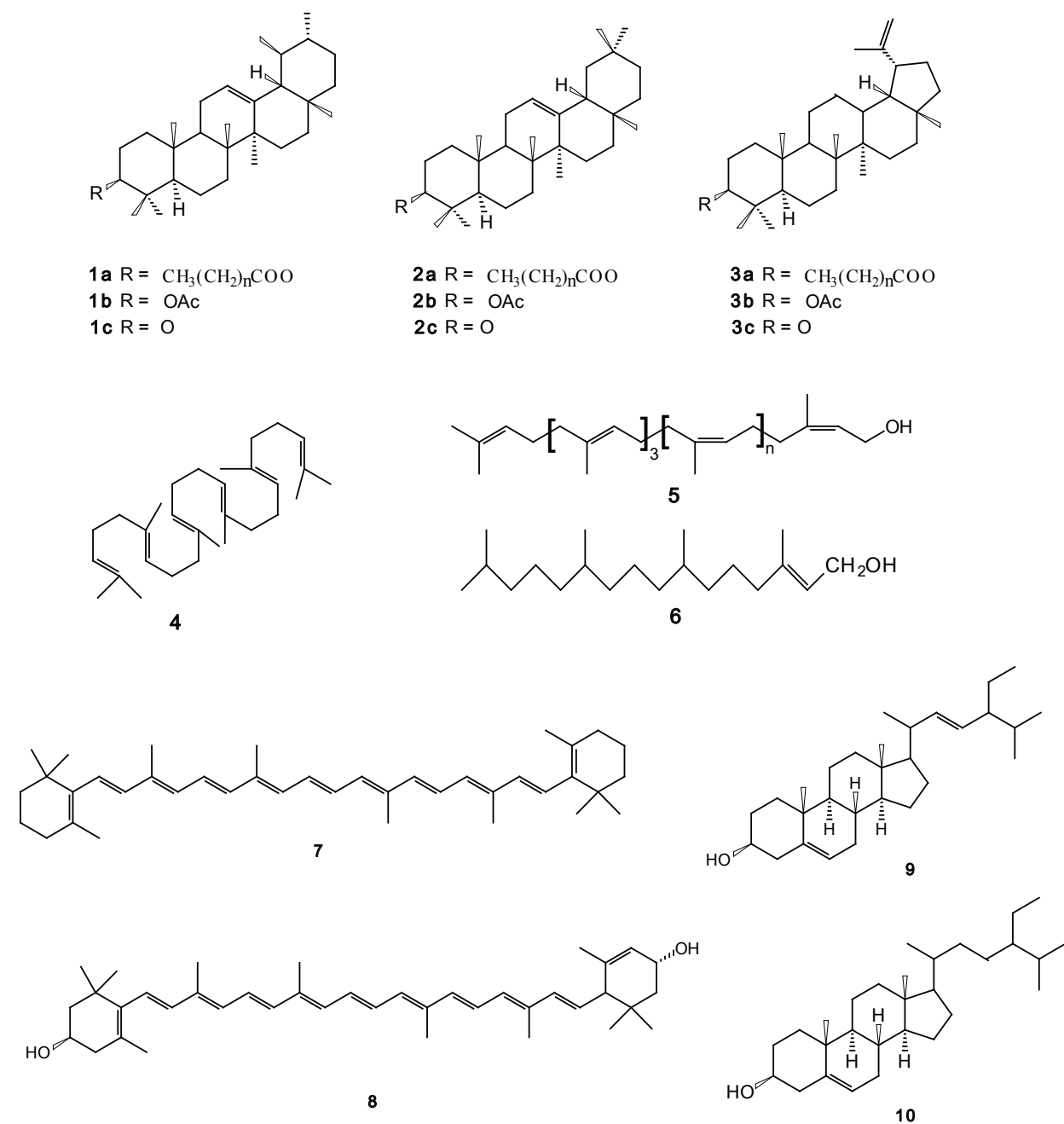


Figure 1. Terpenoids and Sterols from *Ficus ulmifolia* (1-10) and *Ficus pseudopalma* (1-5).

Plant Material

The plant samples were collected from around Lake Buhi, Camarines Sur in September 2007. They were identified as *Ficus pseudopalma* and *Ficus ulmifolia* by Noe Gapas of the Philippine National Museum who also collected the samples. *Ficus pseudopalma* (voucher specimen # 132) and *Ficus ulmifolia* (voucher specimen # 133) were deposited at the Chemistry Department, De La Salle University – Manila.

Isolation

The dichloromethane extract of the air-dried leaves of *F. pseudopalma* (1.9 kg) was chromatographed on a silica gel column using increasing proportions of acetone in dichloromethane at 10% increment. The 10% acetone in dichloromethane fraction was rechromatographed in petroleum ether. The less polar fractions afforded **4** after rechromatography in petroleum ether. The more polar fractions were rechromatographed (7x) in 2.5%

ethyl acetate in petroleum ether. The less polar fractions afforded a mixture of **1c**, **2c**, and **3c**, followed by a mixture of **2a** and **3a**. The more polar fractions were rechromatographed in 5% ethyl acetate in petroleum ether to yield a mixture of **1b** and **2b** from the less polar fractions, while **5** was obtained from the more polar fractions.

The dichloromethane extract of the air-dried leaves of *F. ulmifolia* (742 g) was chromatographed on a silica gel column using increasing proportions of acetone in dichloromethane at 10% increment. The 10% acetone in dichloromethane fraction was rechromatographed in petroleum ether. The less polar fractions afforded **4** and **7**. The more polar fractions were rechromatographed by gradient elution technique. The column was first eluted with 2.5% ethyl acetate in petroleum ether to afford a mixture of **1b**, **2b**, and **3b**, followed by 5% ethyl acetate in petroleum ether to afford a mixture of **1a** and **2a**. The 20% acetone in dichloromethane fraction was rechromatographed in 10% ethyl acetate in petroleum ether to afford **5**. The 40% acetone in dichloromethane fraction was rechromatographed in dichloromethane to afford **6**. The 50% acetone in dichloromethane fraction was rechromatographed in dichloromethane:diethyl ether:acetonitrile (8:1:1) to afford a mixture of **9** and **10** after washing with petroleum ether. The 60% acetone in dichloromethane fraction was rechromatographed in dichloromethane:diethyl ether: acetonitrile (8:1:1) to afford **8** after washing with petroleum ether, then diethyl ether.

RESULTS AND DISCUSSION

The dichloromethane extracts of the air-dried leaves of *Ficus pseudopalma* and *Ficus ulmifolia* afforded terpenoids and sterols by silica gel chromatography. These compounds were identified by comparison of their ^{13}C NMR spectral data with those of α -amyrin fatty acid ester **1a** (Menezes et al. 1998), β -amyrin fatty acid ester **2a** (Menezes et al. 1998; Wang 2007; Barreiros et al. 2002), β -amyrin acetate **2b** (Derome 1987), lupeol fatty acid ester **3a** (Wang 2007), lupeol acetate **3b** (Derome 1987), squalene **4** (Brown & Martens 1977), polyprenol **5** (Rideout et al. 2003), phytol **6** (Ragasa et al. 2003), lutein **7** (Largo et al. 1997), β -carotene **8**, stigmasterol **9**, and sitosterol **10** (Cayme and Ragasa 2004) reported in the literature. The structure of α -amyrin acetate **1b** was deduced by comparison of its ^1H NMR and ^{13}C NMR spectral data with those of α -amyrin fatty acid ester **1a**. Instead of the long chain fatty acid at δ 1.25 for the methylene protons, a methylene triplet at δ 2.30 which

is α to a carbonyl, and a methyl triplet at δ 0.88 for **1a**, an acetoxy methyl was detected at δ 2.01 for **1b**. The ^{13}C NMR spectrum for **1a** gave resonances for the long chain fatty acid methylene carbons centered at δ 29.7, carbonyl carbon of the ester at δ 173.7, and the methyl carbon at the end of the fatty acid chain at δ 14.1. These resonances for **1a** were replaced in **1b** by the resonances for the acetoxy carbons at δ 171.0 and 21.3. Ursenone **1c**, oleanone **2c**, and lupenone **3c** have similar ^1H NMR resonances to α -amyrin acetate **1b**, β -amyrin acetate **2b**, and lupeol acetate **3b**, respectively, except in the regions where their structures differ, e.g., around C-3 where the acetates were replaced by carbonyls. Thus, the resonances at δ 2.01 for the acetoxy methyl and δ 4.40 for the oxymethine protons of **1b**, **2b**, and **3b** were not found in **1c**, **2c**, and **3c**. The ^{13}C NMR resonances for the acetoxy carbons at δ 171.0 and 21.3, and the carbonyl carbon resonances at δ 80.9 for **1b**, **2b**, and **3b** were replaced by the carbonyl carbon resonances at δ 217.9 for **1c**, **2c**, and **3c** (Hisham et al. 1995; Clen et al. 1998).

Although bioassays were not conducted on the isolated compounds, there were previous studies that reported on their biological activities. α -Amyrin acetate has been reported to possess hypoglycemic activity (Narrender et al. 2009), while lupeol exhibited antiulcerolithiatic and diuretic activity (Vidya et al. 2002). α -Amyrin acetate may be one of the active constituents in the anti-diabetes property of *F. pseudopalma*. Lupeol prevented the formation of vesical calculi and reduced the size of the preformed stones in rats (Anand et al. 1994). This indicates that lupeol may be one of the active constituents in the treatment of kidney stones by *F. pseudopalma*. α -Amyrin and β -amyrin were reported to possess anti-inflammatory activity (Recio et al. 1995; Madeiros et al. 2007), while α -amyrin, β -amyrin, and the 3-O-acyl derivatives of α -amyrin and β -amyrin exhibited analgesic property (Otuki et al. 2005; Soldi et al. 2008). Squalene significantly suppresses colonic ACF formation and crypt multiplicity. This strengthens the hypothesis that squalene possesses chemopreventive activity against colon carcinogenesis (Rao et al. 1998). Squalene has cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties (Farvin et al. 2006). Lutein and zeaxanthin may have roles in protecting against age-related macular degeneration (Mozaffarieh et al. 2003). β -Sitosterol has been shown to inhibit proliferation and induce apoptosis in human solid tumors such as colon and breast cancers (Park et al. 2007). Stigmasterol lowers plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Wistar as well as WKY rats (Batta et al. 2006). A polyprenol from the ethanol extract of *Coccinia*

grandis showed significant triglyceride and cholesterol lowering effects in dyslipidemic hamster model (Singh et al. 2007).

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