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One unusual sterol from *Polyscias fruticosa* (L.) Harms (Araliaceae)

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ABSTRACT

A phytochemical study on petroleum ether-diethyl ether (v/v 1:1) extract led to the isolation of one sterol with unusual side chain, 22-dehydro-24-isopropylcholesterol (1) and one common triterpenoid oleanolic acid (2). Compound 1 has been previously identified as a marine invertebrate sterol, here its appearance in terrestrial source of Polyscias fruticosa was first reported. Their structures, including absolute configuration, are elucidated unambiguously by X-ray diffraction, spectroscopic data and comparison with literature.

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1 INTRODUCTION

Polyscias fruticosa (L.) Harms belongs to Araliaceae family and distributes widely in many countries of southeastern Asia and the tropical islands of the Pacific region. In Asian countries, the leaves are used as atonic, anti-inflammatory, antitoxin, and antibacterial. The root is used as a diuretic, febrifuge, antidysentery, and for treatment of neuralgia and rheumatic pains. *P. fruticosa* is also used for other purposes as ornamental plant and spice (Huan *et al*., 1998). The previous phytochemical studies shown that amino acids, polysaccharides, steroids, sesquiterpenoids, triterpenoid saponins, and polyacetylenes are among the components of *P. fruticosa* (Brophy *et al.,* 1990, Lutomski and Luan, 1992, Huan *et al*., 1998, Mahesh, 2008). In this paper, as a part of the search for bioactive compounds from non-polar fraction of *P. fruticosa*, a phytochemical investigation on petroleum ether-diethyl ether (*v/v* 1:1) extract was performed.

2 EXPERIMENT

2.1 Plant material

Polyscias fruticosa (L.) Harms was collected in Tra Vinh province, Vietnam in May 2015. The scientific name was identified by Dr. Dang Minh Quan, Department of Biology, Faculty of Education, Can Tho University, Vietnam. A voucher specimen (No Polys F-0515) was deposited in the herbarium of the Department of Chemistry, Can Tho Univeristy of Medicine and Pharmacy, Vietnam.

2.2 General experimental procedures

The NMR experiments were performed on a Bruker DMX 300 and 500 spectrometers. ESI-HRMS were carried out on a MICROMASS ZabspecTOF spectrometer for electrospray ionization. Melting point was recorded on a Krüss Melting Point Meters M5000. The crystal data was collected on a Enraf-Nonius FR590-kappa diffractometer with a CCD area detector and graphite monochromated MoKα radiation. The structure was solved using direct methods, refined with the Shelx software package, and expanded using Fourier techniques. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in structure factor calculations from their location in difference maps. C bound H atoms were treated as riding in geometrically idealized positions, with Uiso (H) = kUeq (C), where k = 1.5 for the methyl groups, which were allowed to rotate around their C—C bond, and 1.2 for all other C bound H atoms. Computing software for Data Collection, Cell Refinement and Data Reduction using COL-

LECT/HKL2000. For Structure Solution using SHELX-S97 software. For Structure Refinement using SHELXL2012 and CRYSTALBUILDER softwares. For Molecular Graphics using ORTEP-III and MERCURY softwares.

Column chromatography was performed on normal phase silica gel (40-63 μm, Keselgel 60, Merck 7667). Thin layer chromatography was performed on Kieselgel 60F254 plates (Merck), and spots were visualized under UV light or sprayed with vanillin (0.5 g vanillin in 80 mL sulfuric acid and 20 mL ethanol), then heated. All solvents used were purchased from Chemsol, purity ≥ 99.0%.

2.3 Extraction and isolation

Ground dried *P. fruticosa* (30 g) was extracted for 3 hours with 200 mL petroleum ether-diethyl ether (*v/v* 1:1) using magnetic stirrer at room temperature (× 3 times) to furnish 1.30 g of petroleum ether-diethyl ether extract (yield 4.3%).

The extract (1 g) was subjected to silica gel column chromatography using a gradient of solvent *n*-hexane:benzene (10:90 – 0:100) to give 9 fractions. A precipitate occurred in fraction 6. After filtration and recrystallization (in *n*-hexane), compound 1 was obtained (4.20 mg). Fraction 9 was subjected to silica gel column chromatography with *n*-hexane:ethyl acetate (8:2) as eluent to give compound **2** (3.50 mg).

Compound 1: white needles (in $CHCl_3$); R_f = 0.45 (*n*-hexane:chloroform 1:9); M.p 169-172°C; ESI-HRMS *m/z* 427.3956 $[M+H]^+$ (calcd. for $C_{30}H_{51}O$ 427.3939); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta_{H\ ppm}$ 5.35 (1H, *m*, H6), 5.17 (1H, *dd*, *J*= 8.4;15 Hz, H22); 5.03 (1H, *dd*, *J*=8.4;15 Hz, H23); 3.52 (1H, *m*, H3), 1.27 (3H, *s*), 0.72 (3H, *s*); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta_{C\ ppm}$ 71.8 (C3), 140.7 (C5), 121.7 (C6), 138.3 (C22), 129.2 (C23), 25 signals from 56.8 to 12.0 in which three signals were overlapped.

Compound 2: white powder; R_f = 0.50 (chloroform:methanol 95:5); M.p 271-273°C; ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta_{H\ ppm}$ 5.26 (1H, *brs*, H12), 3.21 (1H, *m,* H3); 2.82 (*d*, *J*=10 Hz, H18); 1.13 (3H, *s,* H27); 0.98, 0.93, 0.91, 0.90, 0.77, 0.75 (each 3H, *s*, CH_3 ×6); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta_{C\ ppm}$ 38.5 (C1); 27.7 (C2); 79.1 (C3); 38.8 (C4); 55.3 (C5); 18.3 (C6); 32.5 (C7); 39.3 (C8); 47.7 (C9); 37.1 (C10); 23.4 (C11); 122.7 (C12); 143.6 (C13); 41.7 (C14); 27.2 (C15); 23.0 (C16); 46.6 (C17); 41.1 (C18); 45.9 (C19); 30.7 (C20); 33.8 (C21); 32.7 (C22); 28.1 (C23); 15.6 (C24); 15.3 (C25); 17.1 (C26); 25.9 (C27); 182.9 (C28); 33.1 (C29); 23.6 (C30).

3 RESULTS AND DISCUSSION

From petroleum ether-diethyl ether extract (1.00 g), compound 1 (4.20 mg) was isolated as white needles. The ^{1}H-NMR spectrum of 1 exhibited a pair of double doublets at $\delta_{H\ ppm}$ 5.17 and 5.03 (*J*= 8.4;15 Hz) due to *trans*-oriented olefin protons and a multiplet at $\delta_{H\ ppm}$ 5.35, typical of the olefinic proton of Δ^5-sterols (Kikuchi *et al*., 1982) together with signals arising from a hydroxyl-bearing methine at $\delta_{H\ ppm}$ 3.52 ppm and two tertiary methyl groups at $\delta_{H\ ppm}$ 1.27 and 0.72 (Figure 1). Moreover, the ^{13}C-NMR spectrum showed two signals at $\delta_{C\ ppm}$ 138.3 and 129.2, typical for double bond at C(22)-C(23) of stigmasterol and one oxygenated sp^3 carbon at $\delta_{C\ ppm}$ 71.8 (C3) (Figure 2). In fact, the NMR data of 1 was very similar to that of stigmasterol, a common sterol previously reported in many plant. However, the ESI-HRMS showed a peak at *m/z* 427.3956 $[M+H]^+$ corresponding to formula $C_{30}H_{50}O$ suggesting an unusual 24-isopropyl steroid skeleton.

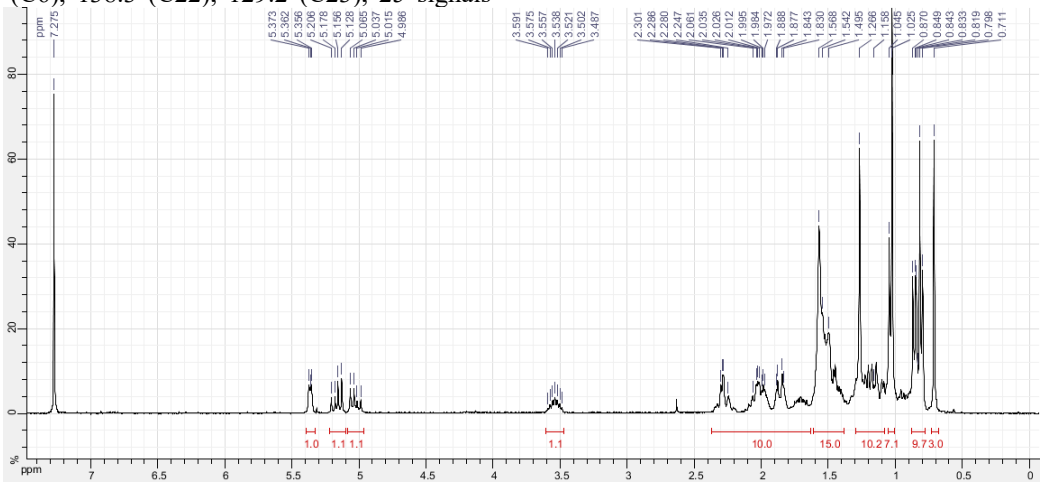


Fig. 1: ^{1}H-NMR spectrum of 1 ($CDCl_3$, 300 MHz)

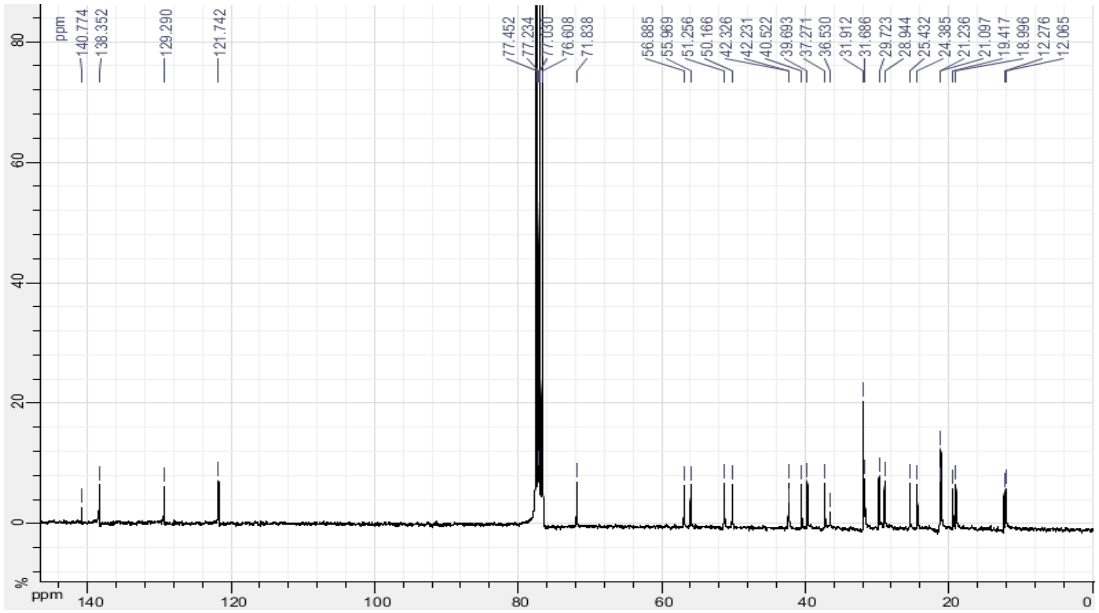


Fig. 2: ^{13}C-NMR spectrum of 1 ($CDCl_3$, 75 MHz)

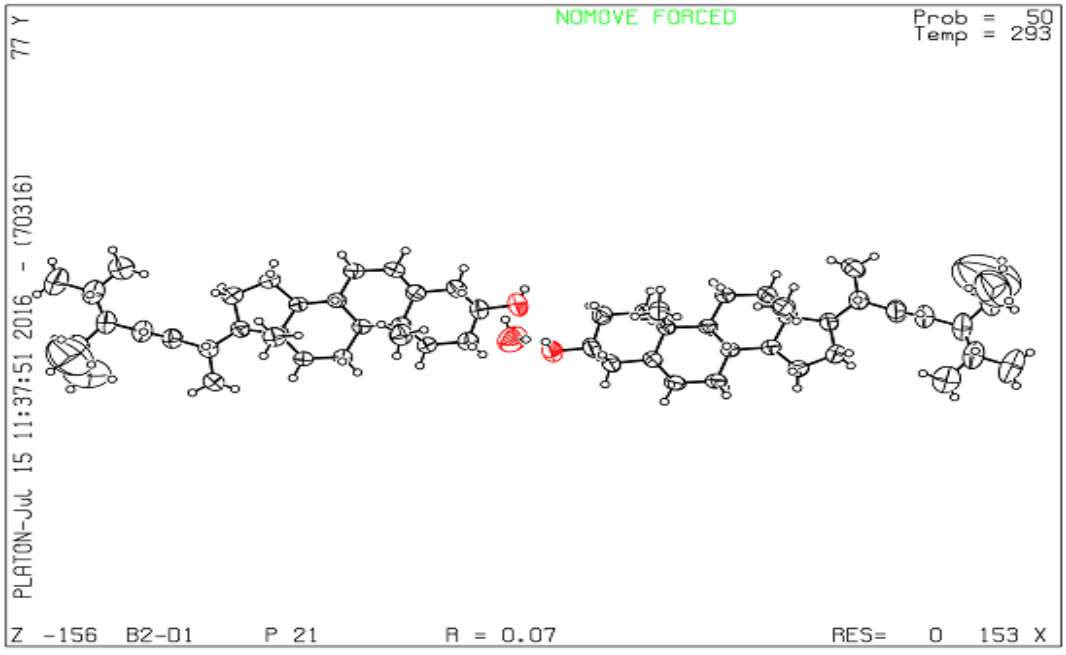
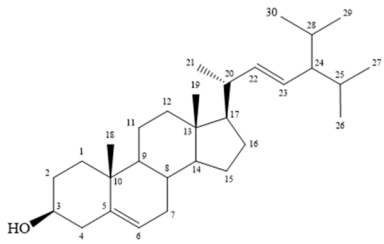


Fig. 3: Structure and absolute configuration of 1 by X-ray

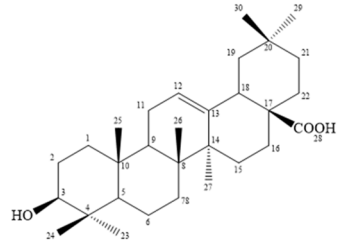
In this study, the structure and stereochemistry of 1 was unambiguously determined to be 22-dehydro-24-isopropylcholesterol by X-ray diffraction (Figure 3). Interestingly, in its crystal structure, two sterol molecules are held together by one water molecule via hydrogen bond. In nature, steroids with an additional isopropyl group appended at C-24 are relatively rare. The first such compounds were reported in 1979 from marine sponges belonging to the genera *Pseudaxinyssa* and *Verongia* (Dai *et al*., 2010). Here, 22-dehydro-24-isopropylcholesterol was reported the first time from terrestrial source *P. fruticosa*.

Compound 2 (3.50 mg) was obtained as a white powder. The ^{1}H-NMR spectrum of 2 showed seven tertiary methyl groups at $\delta_{H\ ppm}$ 0.75, 0.77, 0.90, 0.91, 0.93, 0.98 and 1.13 on an oleanane skeleton. A doublet of one proton at $\delta_{H\ ppm}$ 2.82 and broad singlet of one vinyl proton at $\delta_{H\ ppm}$ 5.26 were assigned to H18 and H12, respectively, suggesting an olean-12-ene skeleton. One methine proton at $\delta_{H\ ppm}$ 3.21 (*m*) showed that 2 has at least one hydrox-

yl group. The ^{13}C-NMR spectrum exhibited thirty signals with one carboxyl group at δ_{ppm} 182.9 (C28), two typical olefinic carbons at δ_{ppm} 122.7 (C12) and 143.6 (C13), one oxygenated carbon (C3) at δ_{ppm} 79.1. The spectral data were similar to those of oleanolic acid (Gohari *et al.*, 2009).



22-Dehydro-24-isopropylcholesterol (**1**)



Oleanolic acid (**2**)

Fig. 4: Structures of isolated compounds from *P. fruticosa*

4 CONCLUSIONS

From the petroleum ether-diethyl ether (*v/v* 1:1) extract of *P. fruticosa*, one unusual sterol 22-dehydro-24-isopropylcholesterol 1 and oleanolic acid 2 were isolated. Their structures, especially absolute configuration of 1, were determined clearly by spectroscopic methods NMR, ESI-HRMS and X-ray diffraction. Further studies on chemical constituents of *P. fruticosa* are in progress.

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