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Studies on The Chemical Constituents of Bark Roots of *Phyllanthus columnaris*

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Fractionation of the ethyl acetate extracts of the bark roots of *Phyllanthus columnaris* of the Euphorbiaceae family, has led to the isolation and structural elucidation of three different triterpenoids. The separations of the chemical components were carried out by different chromatographic techniques and their structures were elucidated by spectroscopic methods including nuclear magnetic resonance as well as mass spectrometry. Three compounds were isolated and identified; that are lupeol acetate, lupeol and friedelin.

Key Words: Euphorbiaceae, *Phyllanthus columnaris*, Lupeol acetate, Lupeol, Friedelin, NMR.

INTRODUCTION

Plants of the genus *Phyllanthus* are part of the Euphorbiaceae family. Comprising more than 500 species, *Phyllanthus* are widely distributed throughout South Africa and Asia. Many of which are used medicinally in different countries. Euphorbiaceae, a common rainy season weed, is found in both cultivated fields and wastelands in India. It is an annual herb with height verging between 30 and 60 cm. Its roots, leaves, fruits, milky juice and whole plants are used as medicine[1]. Fruits are useful for tubercular ulcers, wounds, sores, scabies and ring worm[2]. In this paper, the isolation and characterization of three known compounds from *Phyllanthus columnaris* are reported.

EXPERIMENTAL

TLC and preparative TLC were performed using pre-coated aluminium and glass plates with silica gel 60 F_{254}, whereas column chromatography was carried out on silica gels 230-400 mesh. Spots and bands for compounds on TLC were detected using UV light. UV spectra were recorded on a UV1650PC spectrophotometer. Bruker SMART APEX and the accompanying SHELXTL programming suite carried out X-ray structure determination. Proton NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Jeol JNM-ECP400 and chemical shifts in ppm were referenced to internal acetone-d_6 and $CDCl_3$, respectively. ^1H-^1H COSY and NOESY spectra were acquired using the standard Joel software.

Plant material: The bark roots of *Phyllanthus columnaris*, which were collected from pula Langkawi Voucher specimens of WAY131 have been deposited at the Herbarium of Universiti Kebangasaan Malaysia.

Extraction and isolation: The air-dried powder bark roots (300 g) of *Phyllanthus columnaris* were extracted from ethyl acetate (3 d) and the combined extracts evaporated to give a brown gummy residue (5.5 g). 2.5 g of the extract was subjected to silica gel flash column chromatography (FCC) with hexane containing increasing percentages of ethyl acetate as eluent and each collected fraction was 20 mL. Fractions 2-8 were combined and rechromatographed by radial chromatography to yield three compounds: (4.0 mg) of a compound which is identified as lupeol acetate (**1**, Fig. 1), R_f 0.65 (hexane-EtOAc); (4.1 mg) of a compound that is identified as lupeol (**2**, Fig. 2), R_f 0.7 (hexane-EtOAc) and the last constituent is identified as friedelin (Fig. 3) (3.1 mg), R_f 0.73 (hexane-EtOAc). Lupeol acetate, lupeol and friedelin were identified by comparison with data from previous NMR and mass spectra[3-5].

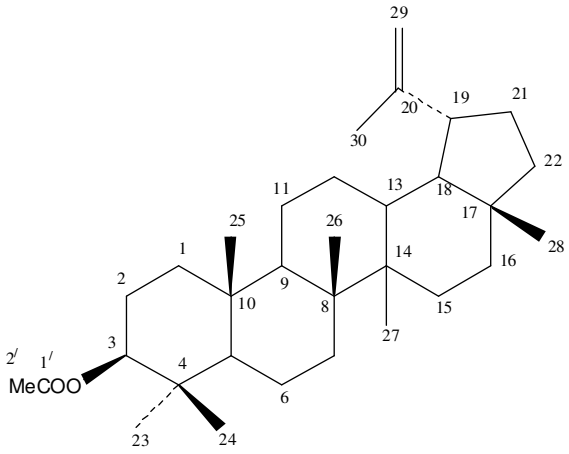

Fig. 1. Structure of lupeol acetate

Lupeol acetate (1): White needles (3.6 mg). EIMS for $C_{32}H_{52}O_2$ m/z (rel. int.): 468 [M^+] (17.2 %), 453 (2.9 %), 408 (1.7 %), 357 (3.9 %), 218 (15.2 %), 189 (46.4 %), 109 (29.1 %), 43 (100 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.69 (1H, s, H-29b), 4.57 (1H, s, H-29a), 4.47 (1H, dd, J = 4.4, 12.8 Hz, H-3), 2.05 (3H, s, H-2′), 1.69 (3H, s, H-30), 0.94 (3H, s, H-28), 0.85 (3H, s, H-23), 0.84 (3H, s, H-24), 0.83 (3H, s, H-26), 0.79 (3H, s, H-27). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.3 (C-1'), 151.2 (C-20), 109.6 (C-29), 81.2 (C-3), 55.6 (C-5), 50.5 (C-9), 48.5 (C-18), 48.2 (C-19), 43.2 (C-17), 43.0 (C-14), 41.0 (C-8), 40.2 (C-22), 38.6 (C-1), 38.0 (C-4), 37.3 (C-10), 36.2 (C-13), 35.8 (C-16), 34.4 (C-7), 30.0 (C-21), 28.2 (C-2'), 27.6 (C-23), 25.3 (C-15), 24.0 (C-12), 21.7 (C-2), 21.1 (C-11), 19.5 (C-30), 18.4 (C-6), 18.2 (C-28), 16.7 (C-24), 16.4 (C-25), 16.2 (C-26), 14.7 (C-27).

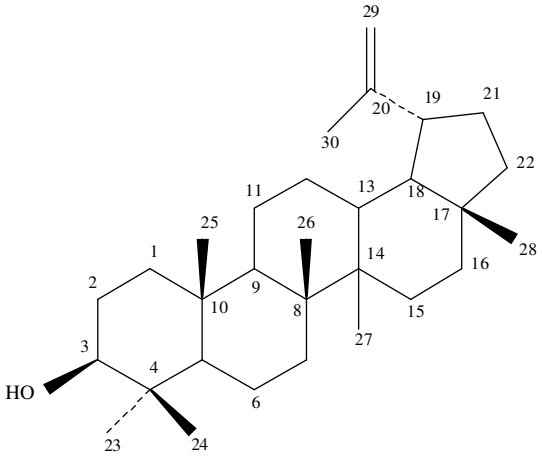

Fig. 2. Structure of lupeol

Lupeol (2): White powder (3.1 mg), m.p. 215-216 ºC. EIMS for $C_{30}H_{50}O$ m/z (rel. int.): 426 [M^+] (33.4 %), 365 (14.5 %), 207 (51.3 %), 189 (25.8 %), 161 (22.9 %), 135 (71.0 %), 107 (100 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.68, 4,56 (2H, s, H-29a, b), 3.16 (1H, dd, J = 4.76, 11.00 Hz), 0.75, 0.78, 0.82, 0.93, 0.95, 1.02, 1.25 (each 3H, s, Me×7). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 151.1 (C-20), 109.5 (C-29), 79.1 (C-3), 55.5 (C-5), 50.6 (C-9), 48.5 (C-18), 48.1 (C-19), 43.2 (C-17), 43.0 (C-14), 41.0 (C-8), 40.2 (C-22), 39.0 (C-13), 38.9 (C-4), 38.2 (C-1), 37.3 (C-10), 35.8 (C-16), 34.5 (C-7), 30.0 (C-21), 28.2 (C-23), 27.6 (C-15), 27.5 (C-12), 25.3 (C-2), 21.1 (C-11), 19.5 (C-30), 18.5 (C-6), 18.2 (C-28), 16.3 (C-25), 16.2 (C-26), 15.6 (C-24), 14.7 (C-27).

Friedelin (3): White powder (3.5 mg), m.p. 259-262 ºC. EIMS for $C_{30}H_{50}O$ m/z (rel. int.): 426 [M^+]. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.73, 0.87, 0.89, 0.96, 1.00, 1.00, 1.05, 1.18 (each 3H, s, Me×8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 213.6 (C-3), 59.7 (C-10), 58.5 (C-4), 53.3 (C-8), 43.0 (C-18), 42.4 (C-5), 41.8 (C-2), 41.5 (C-6), 39.9 (C-15), 39.5 (C-22), 38.5 (C-14), 37.6 (C-9), 36.2 (C-16), 35.8 (C-11), 35.6 (C-19), 35.3 (C-30), 32.9 (C-15), 32.7 (C-21), 32.3 (C-8), 32.0 (C-29), 30.7 (C-12), 30.3 (C-17), 28.4 (C-20), 22.5 (C-1), 20.5 (C-26), 18.9 (C-27), 18.5 (C-7), 18.1 (C-25), 14.8 (C-24), 7.1 (C-3).

RESULTS AND DISCUSSION

The concentrated ethyl acetate extract of the bark roots of *Phyllanthus columnaris* was repeatedly chromatographed over silica gel flash column chromatography and compounds **1-3** were eluted in the order of increasing polarity. The ^{1}H and ^{13}C NMR spectral data for these compounds revealed that **1** and **2** belong to the lupine group. Compound **3** was identified as friedelin from its physical constants and spectral data.

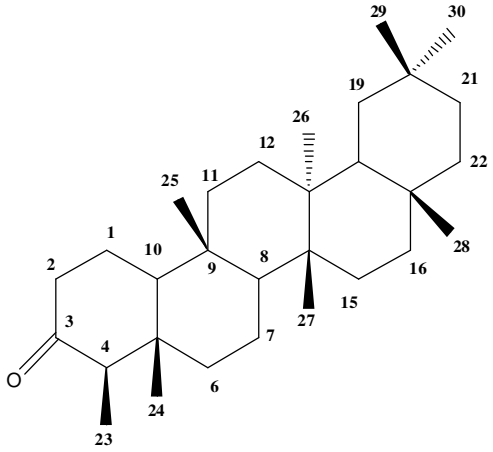

Fig. 3. Structure of friedelin

Compound **1** was isolated as white needles. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) showed the presence of eight tertiary methyl singlets at δ 0.79, 0.83, 0.84, 0.85, 0.94, 1.03, 1.69 and 2.05. Two protons appeared at δ 4.57 and 4.69 as singlets, representing the exocyclic double bond protons H-29a and H-29b. And ^{13}C NMR spectrum showed a carbonyl group signal at δ 171.42, also C-3 appeared at δ 81.1 and the alkene carbons appeared at δ 151.20 and 109.57. Lupeol acetate has never been isolated before from *Phyllanthus reticulatus*. It was found in *Deertongue* leaf[6], *Erythroxylum Leal costae*[7], stem-bark of *Artocarpus chaplasha*[8] and *Ficus hispida*[9].

Compound **2** is a pentacyclic triterpene. It was white powder. The EI-mass spectrum of **2** showed the molecular ion at m/z 426 [M^+] corresponding to the formula $C_{30}H_{50}O$ and in agreement with other spectroscopic data. The ^{1}H NMR spectrum showed seven tertiary methyl singlets and one secondary hydroxyl group. It also showed olefinic protons at δ 4.68 and 4.54. ^{13}C NMR of the compound showed 30 signals for the terpenoid of lupine skeleton which was represented by seven methyl groups. The carbon bonded to the hydroxyl group C-3 appeared at δ 79.1, while the alkenic carbons appeared at δ 151.1 and 109.5. The presence of lupeol in the *Phyllanthus reticulatus* was not reported before the current study. The lupeol was reported earlier from the seeds of bark of *Heritiera utilis*[10] and *Euphorbia lateriflora*[11].

Compound **3** was isolated as white powder, m.p. 259-262 ºC. The mass spectrometry indicated its molecular formula as $C_{30}H_{50}O$ ([M]$^+$ m/z 426). There was no significant UV absorption but the IR spectrum revealed a band at 1715 cm^{-1} for the presence of carbonyl group. The ^{1}H NMR spectrum showed eight methyl groups, all of which were singlets except for one at δ 0.87, typical of a C-4 methyl in a friedelane type triterpene. The spectrum further exhibited a pair of deshielded methylene proton at δ 2.27 and δ 2.25. The ^{13}C NMR of the compound showed 30 signals for the friedelane which was represented by eight methyl groups. Carbon bonded to the

carbonyl group C-3 appeared at δ 213.6. The presence of friedelin in the *Phyllanthus reticulatus* was not reported before the present study. The friedelin was reported earlier from the leaves of *Phyllanthus reticulatus*[12], *Tovomita brasiliensis*[13] and *Hypericum ascyron*[14].

Conclusion

The isolation and identification of lupeol acetate (**1**), lupeol (**2**) and friedelin (**3**) from the bark roots of *Phyllanthus columnaris* was reported from this plant. The work was carried out by means of various physical (solvent extraction, radial chromatography) and spectral techniques.

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