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Terpenoids from *Dacrycarpus imbricatus*

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Abstract

The phytochemical investigation of the hexane extract from the twigs and leaves of *Dacrycarpus imbricatus* (Blume) de Laub led to the isolation of a rare sesquiterpene, spathulenol (**1**) along with three diterpenes named pimaric acid (**2**), *trans*-communic acid (**3**) and *cis*-communic acid (**4**). Their structures were determined by combination of spectral analysis and comparison with reported data. This is the first report on isolation of compound **1** from the Podocarpaceae family.

Keywords. *Dacrycarpus imbricatus*, spathulenol, pimaric acid, communic acid.

1. INTRODUCTION

Dacrycarpus imbricatus (Blume) de Laub (Podocarpus kawaii Hayata) is a coniferous tree, up to 40 m tall with red brown bark, belonging to Podocarpaceae family. It is mainly distributed in the West Pacific islands near China and Viet Nam. This tree is classified as 'Least Concern' in the IUCN Red List of Threatened Species (2011) [1]. Its timber had been used in construction, for furniture, firewood and in some areas cultivated as ornamental tree. In our previous paper, we described the isolation and identification of 20-hydroxyecdysone from the bark of *D. imbricatus* and its effect on OCI-AML cell proliferation in vitro [2]. In the course of our continuing search for the bioactive constituents of this plant, a rare sesquiterpene, spathulenol (**1**) and three diterpenes:pimaric acid (**2**), mixture of *trans*- and *cis*-communic acid (**3**+**4**) were isolated from the twigs and leaves of *D. imbricatus*. Their structures were elucidated by the spectroscopic means and comparison with published data.

2. EXPERIMENTAL

2.1. Materials and methods

D. imbricatus was collected in Lam Dong province, Viet Nam (August, 2012) and identified by Dr. Nguyen Tien Hiep (Vietnam National Museum of Nature). A voucher specimen (VNMN. B0000050010) was deposited in the Vietnam National Museum of Nature, Vietnam Academy of Science and Technology (VAST).

ESI-MS: Agilent LC-MSD-Trap SL. ^{1}H-NMR (500.13 MHz) and ^{13}C-NMR (125.77 MHz) spectral data were measured on a Bruker Avance 500 Ultrashield NMR Spectrometer at 25 ^{o}C. Chemical shifts were reported as δ values with reference to TMS as internal standard for ^{1}H (δ = 0 ppm) and $CDCl_3$ for ^{13}C (δ = 77.0 ppm). TLC: Silica gel 60 F_{254} (0.25mm, Merck); reversed phase $RP_{18}F_{254S}$ (0.25mm, Merck). CC: Silica gel 60 (230-400 mesh, Merck) for the first column, silica gel 60, 40-63 μm (Merck) for the following columns.

2.2. Extraction and isolation

The dried ground twigs and leaves of *D. imbricatus* (900 g) were extracted with methanol-water (90:10, v/v) at room temperature (4 times x 3 lit, each overnight). After concentration under reduced pressure, the obtained crude extract (62.2 g) was

suspended in water (150 ml) and then sequentially partitioned with *n*-hexane, ethyl acetate and *n*-butanol. The organic solvents were evaporated to yield the corresponding extracts (10.8 g, 18.0 g and 10.2 g, respectively). The condensed *n*-hexane extract (10.8 g) was separated on silica gel column, eluting with a gradient of *n*-hexane-EtOAc (100:0 → 80:20) to yield 40 fractions (FH1–FH40) on the basis of TLC analysis. Fraction FH 22 was further purified on a silica gel column eluted with *n*-hexane-EtOAc (6:4, v/v) to furnish compound **1** (10 mg). Recrystallization of the precipitated solid from fraction FH 31 with cold methanol gave compound **2** (30 mg). The mixture of compounds **3**+**4** (20 mg) was obtained from FH3 by column chromatography on silica gel eluted with hexane:EtOAc (9:1 → 1:1).

2.3. Spectral data of isolated compounds

Spathulenol (1): Colorless semisolid. ESI-MS *m/z*: 221.3 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 500 MHz): 4.69 (1H, *s*, H-14a), 4.66 (1H, *s*, H-14b), 2.42 (1H, *dd*, *J* = 13.0, 6.0 Hz, H-4a), 2.20 (1H, *m*, H-6), 2.06 (1H, *d*, *J* = 13.0 Hz, H-4b), 1.77 (1H, *m*, H-8a), 1.55 (1H, *m*, H-8b), 1.32 (1H, *d*, *J* = 10.5 Hz, H-10), 1.28 (3H, *s*, H-15), 1.06 (3H, *s*, H-12), 1.03 (3H, *s*, H-13), 0.71 (1H, *ddd*, *J* = 11.0, 9.5, 6.0 Hz, H-2), 0.47 (1H, *dd*, *J* = 11.0, 9.5 Hz, H-1). ^{13}C-NMR ($CDCl_3$, 125 MHz): 153.46 (C-5), 106.26 (C-14), 80.99 (C-9), 54.35 (C-10), 53.41 (C-6), 41.75 (C-8), 38.87 (C-4), 29.92 (C-1), 28.66 (C-12), 27.50 (C-2), 26.72 (C-7), 26.08 (C-15), 24.79 (C-3), 20.27 (C-11), 16.34 (C-13).

Pimaric acid (2): Colorless semisolid. ESI-MS *m/z*: 303.6 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 500 MHz): 5.77 (1H, *dd*, *J* = 17.0, 10.5 Hz, H-15), 5.22 (1H, s, H-14), 4.90 (1H, *dd*, *J* = 17.0, 1.5 Hz, H-16a), 4.88 (1H, *dd*, *J* = 10.5, 1.5 Hz, H-16b), 1.21 (3H, *s*, H-19), 1.04 (3H, *s*, H-17), 0.84 (3H, *s*, H-20). ^{13}C-NMR ($CDCl_3$, 125 MHz): 184.92 (C-18), 148.91 (C-15), 136.63 (C-8), 129.13 (C-14), 110.16 (C-16), 50.58 (C-9), 48.84 (C-5), 47.28 (C-40, 38.30 (C-1), 37.74 (C-10), 37.40 (C-13), 37.05 (C-3), 35.48 (C-7), 34.46 (C-12), 26.04 (C-17), 24.91 (C-6), 18.57 (C-11), 18.16 (C-2), 16.78 (C-19), 15.22 (C-20).

***Trans*- and *cis*-communic acid (3+4)**: Semisolid. ESI-MS *m/z*: 303.1 $[M+H]^+$, ratio of *trans*- and *cis*-isomers ≈ 4:1).

Trans-communic acid **(3)**: ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.32 (1H, *dd*, *J* = 17.0, 10.5 Hz, H-14), 5.41 (1H, *t*, *J* = 6.0 Hz, H-12), 5.04 (1H, *d*, *J* = 17.0 Hz, H-15a), 4.88 (1H, *d*, *J* = 10.5 Hz, H-15b), 4.84 (1H, *s*, H-17a), 4.47 (1H, *s*, H-17b), 1.75 (3H, *s*, H-16), 1.25 (3H, *s*, H-18), 0.65 (3H, *s*, H-20). ^{13}C-NMR ($CDCl_3$, 125 MHz): 183.90 (C-19), 147.93 (C-8), 141.61 (C-12), 133.90 (C-14), 133.45 (C-13), 109.91 (C-15), 107.66 (C-17), 56.43 (C-5), 56.29 (C-9), 44.21 (C-4), 40.37 (C-10), 39.27 (C-6), 38.49 (C-1), 37.93 (C-3), 29.04 (C-18), 25.84 (C-7), 23.31 (C-11), 19.93 (C-2), 12.83 (C-20), 11.84 (C-16).

Cis-communic acid (**4**): ^{1}H-NMR ($CDCl_3$, 500 MHz): 6.78 (*dd*, *J* = 17.5, 11.0 Hz, H-14), 5.31 (*t*, *J* = 6.0 Hz, H-12), 5.18 (*d*, *J* = 17.5 Hz, H-15a), 5.08 (*d*, *J* = 11.0 Hz, H-15b), 4.84 (*s*, H-17a), 4.49 (*s*, H-17b), 1.77 (*s*, H-16), 1.25 (*s*, H-20), 0.65 (*s*, H-20). ^{13}C-NMR ($CDCl_3$, 125 MHz): 183.90 (C-19), 147.93 (C-8), 133.86 (C-14), 131.67 (C-12), 131.56 (C-13), 113.25 (C-15), 107.80 (C-17), 56.70 (C-9), 56.43 (C-5), 44.21 (C-4), 40.43 (C-10), 39.27 (C-6), 38.52 (C-1), 37.93 (C-3), 29.04 (C-18), 25.84 (C-7), 22.28 (C-11), 19.93 (C-2), 19.72 (C-16), 12.83 (C-20).

2.4. Bioactivity tests

Cytotoxicity tests were performed according to Likhiwitayawuid et al. [9] and Skehan et al. [10] at different concentrations in 96-well plates. The KB and Hep-G2 cell lines were maintained in RPMI-1640 culture medium with 10 % fetal bovine serum (FBS). Ellipticine was used as positive control.

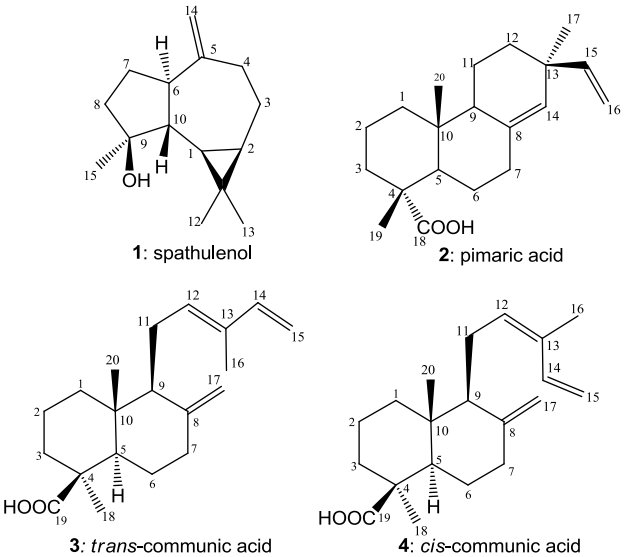

Figure 1: Chemical structures of isolated compounds **1–4** from *Dacrycarpus imbricatus*

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless semisolid. Its molecular formula, $C_{15}H_{24}O$, was deduced from combined analysis of ion peak at *m/z* 221.3 $[M+H]^+$ in the positive ESI MS and ^{1}H-, ^{13}C-NMR and DEPT spectral data. ^{13}C-NMR and HSQC spectral data of **1** confirmed the presence of 15 carbons, including three methyls, five methylenes, four methines and three quaternary carbons (in which has

one carbon bonded to oxygen atom). The ^{1}H-NMR spectrum showed the presence of three methyl groups bonded to quaternary carbons at δ_H 1.28 (3H, *s*, H-15), 1.06 (3H, *s*, H-12), 1.03 (3H, *s*, H-13), an exocyclic methylene at δ_H 4.69 (1H, *s*, H-14a), 4.66 (1H, *s*, H-14b), a cyclopropyl moiety at δ_H 0.71 (1H, *ddd*, *J* = 11.0, 9.5, 6.0 Hz, H-2), 0.47 (1H, *dd*, *J* = 11.0, 9.5 Hz, H-1). Two typical cyclopropyl protons are characteristic of an aromadendrane-type and these data are in accordance with a sesquiterpenoid [3, 4]. In the HMBC experiment, the correlations between H_2-14 and C-4, C-5, C-6; H-10; H_2-8 with C-9 was presented, suggesting the exocyclic methylene and OH groups was located at C-5, C-9, respectively. The nuclear overhauser effect (NOEs) between the methine proton H-1 and the methyl protons H-15 implied that the stereochemistry of the methyl and hydroxyl groups at C-9 were *α*- and *β*-oriented, respectively. Combined all the above spectroscopic data and comparison with literature data [4], the structure of **1** is identified as spathulenol. This compound is a rare sesquiterpene which was previously isolated from the plant *Eucalyptus spathulata* [5], but this is the first time it has been found in the Podocarpaceae family [3-5].

Compound **2** was isolated as a semisolid. The molecular formula of **2** was assigned as $C_{20}H_{30}O_2$ by the molecular ion peak at *m/z* 303.2 $[M+H]^+$ in the positive ESI-MS and NMR data. ^{1}H NMR spectrum of **2** showed a terminal vinyl group [δ_H 5.77 (1H, *dd*, *J* = 17.0, 10.5 Hz, H-15), 4.90 (1H, *dd*, *J* = 17.0, 1.5 Hz, H-16a), 4.88 (1H, *dd*, *J* = 10.5, 1.5 Hz, H-16b); δ_C 148.91 (C-15), 110.16 (C-16)], a trisubstitued olefin group (δ_H 5.22 (1H, *s*, H-14); δ_C 136.63 (C-8), 129.13 (C-14)] and three methyl groups at δ_H 1.47, 1.60, 2.37 (each 3H, s). Except for these moieties the ^{13}C-NMR and HSQC showed the resonances of a carboxyl group at δ_C 184.94, seven methylenes, two methines and three quaternary carbons. These data suggested that **2** is a pimarane diterpenoid. By means of its spectrometric analysis and comparison with previously published data [6], compound **2** was determined as pimaric acid.

Trans- and *cis*-communic acid (**3+4**) were isolated as a mixture. In the ^{1}H- and ^{13}C NMR spectra showed many quite similar pairs of signals in chemical shifts and shape of two compounds with a ratio of about 4:1. The ESI-MS showed one molecular ion peak at *m/z* 303.1, which indicated this is a mixture of two isomers. The ^{1}H-NMR spectrum exhibited signals for three methyl groups at δ_H 1.75/1.77, 1.25 and 0.65/0.64 and an exomethylene at δ_H 4.84/4.87 and 4.47/4.49 (4×s), which were characteristic of a labdane-type diterpene. Additionally it exhibited downfield signals for an olefinic protons at δ_H 5.41/5.31 (*t*, *J* = 6.0 Hz), a vinylic group at δ_H 6.32 (1H, *dd*, *J* = 17.0, 10.5 Hz/6.78 (*dd*, *J* = 17.5, 11.0 Hz); 5.04 (1H, *d*, *J* = 17.0 Hz), 4.88 (1H, *d*, *J* = 10.5 Hz)/5.18 (*d*, *J* = 17.5 Hz), 5.08 (*d*, *J* = 11.0 Hz). Comparison of their NMR data with those reported in the literatures [7, 8], **3** and **4** were determined as *trans*- and *cis*-communic acid, respectively.

The cytotoxic activity of of the major pure compound (**2**, 30 mg) was evaluated against the two human cancer cell lines KB (*mouth epidermal carcinoma*) and HepG2 (*hepatocellular carcinoma*). Compound **2** was medium active against both tested cancer cell lines with IC_{50} values of 173.8 and 99.6 μM, respectively.

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