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Triterpenoid cinnamate, acetate and palmitate from *Hoya kerrii* leaves

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ABSTRACT

Two new triterpenoid cinnamates, namely, *3β-O-D-friedours-11α,12α-epoxy-14-enyl cinnamate* and 3β-*O*-29-*nor*-20-oxolupanyl cinnamate, as well as thirteen known compounds were isolated from hexanes extract of the leaves of *Hoya kerrii.* The structural elucidation of the compounds was performed using spectroscopic methods. Among several isolated triterpenoid cinnamates, lupeol cinnamate is known to possess strong anti-inflammatory properties. The presence of 3β-*O*-D-friedours-11α,12α-epoxy-14-enyl and 3β-*O*-D-friedoolean-11α,12α-epoxy-14-enyl esters has only been reported in a few species of Apocynaceae.

1. Introduction

Hoya kerrii Craib. [syn. *H. obovata* Decne. *var. kerrii* (Craib.) Costantin] of the Apocynaceae family is known in Thailand as “Dang and Tang” (Smitinand, 2014). Its latex, stems, and leaves have been reported to possess wound healing and anti-inflammatory properties. We have recently reported the isolation of some anti-inflammatory steroid glycosides with new skeletons from the stems of this plant (Seeka et al., 2017). Further investigation of the hexanes leave extract, which inhibited NO production in RAW264.7 cells with an IC_{50} value of 93.6 μg/mL and showed several fluorescent spots on TLC chromatograms, has led to the isolation of two new triterpenoid cinnamates (**1** and **2**) in addition to 13 known compounds identified as 3β-*O*-D-friedoolean-11α,12α-epoxy-14-enyl cinnamate (**3**) (Lai et al., 1987), β-sitosteryl-3β-glucopyranoside 6′-palmitate (Nguyen et al., 2004; Kuo et al., 1998), phytol, 3β-*O*-D-friedoolean-11α,12α-epoxy-14-enyl palmitate (Barreiros et al., 2002; Huang et al., 1990), 3β-*O*-D-friedoolean-11α,12α-epoxy-14-enyl acetate (marsformoxide B) (Ito and Lai, 1979, 1978), lupeol cinnamate (Wood et al., 2001; Akihisa et al., 2010), α-amyrin cinnamate (Wood et al., 2001; AKihisa et al., 2010), β-amyrin cinnamate (Akihisa et al., 2010), a mixture of β-sitosterol and stigmasterol, citrostadienol (Rao and Suseela, 1982; Yan et al., 2015; Zhang et al., 2006), a ceramide [(2S,3S,4R)-2-[(2R)-2-hydroxytetracosanoylamino]-1,3,4-octadecanetriol] (Zeng et al., 2012), lupeol and 1,2-di-hexadecanoyl-3-(9*Z*-octadecenoyl)-*sn*-glycerol. Herein, we report the spectroscopic identification of **1** and **2**. Because there was no available physical data or ^{1}H and ^{13}C NMR spectroscopic data for **3**, although this compound was documented to be isolated from *Gymnema alternifolium* (Lai et al., 1987), that data was also included in this report.

2. Results and discussion

Compound **1** was obtained as a colourless solid, m.p. = 229–230 °C, with a molecular formula of $C_{39}H_{55}O_3$ based on its HRESIMS $[M+H]^+$ ion at m/z = 571.4156 (calcd for $C_{39}H_{56}O_3$: 571.4137). The IR spectrum showed cinnamoyl absorption bands at ν_{max} = 1708 and 1275 cm^{-1}, an alkene band at 1638 cm^{-1} and an epoxide ring band at 891 cm^{-1} (Fig. 1). The ^{1}H NMR spectrum of **1** exhibited characteristic proton resonances of a cinnamoyl group at δ_H = 7.65 (1H, d, *J* = 16.0 Hz, H-7′), 7.36 (2H, H-3′) and 7.35 (1H, H-4′), 7.51 (2H, dd, *J* = 5.6 and 3.4 Hz, H-2′ and H-6′), 6.43 (1H, d, *J* = 16.0 Hz, H-8′), in addition to a trisubstituted olefinic proton resonance at δ_H = 5.55 (1H, dd, *J* = 8.1 and 2.9 Hz). The ^{13}C NMR spectrum exhibited 37 carbon resonances, comprised of eight methyls, seven methylenes, 14 methines (including three aromatic, three olefinic and three oxygenated methines), one carbonyl and seven quaternary carbons (including one olefinic and one aromatic). The ^{1}H NMR spectrum showed six tertiary methyls (δ_H = 0.88–1.11) and two secondary methyl groups at δ_H = 1.10 (d, *J* = 5.5 Hz) and 0.98 (d, *J* = 6.7 Hz). The presence of two shielded and mutually coupled oxymethine proton resonances at δ_H = 3.16 (dd, *J* = 6.0 and 4.8 Hz, H-11) and 2.99 (d, *J* = 4.8 Hz, H-12) with two shielded oxymethine carbon resonances at δ_C = 53.7 (C-11)

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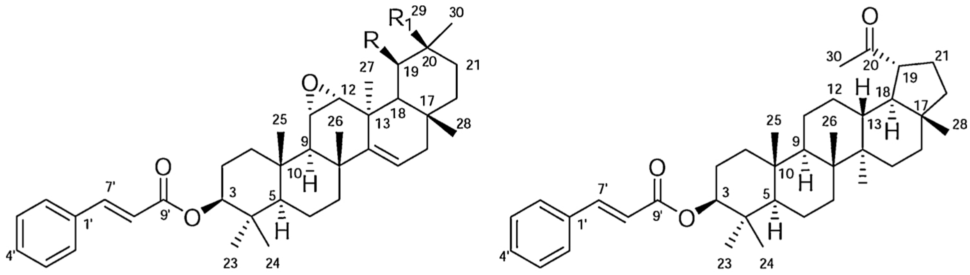
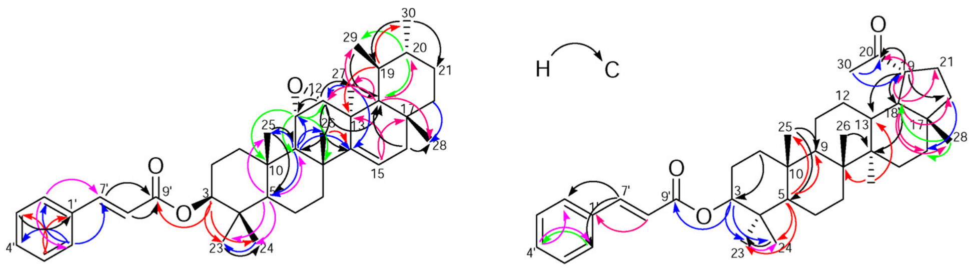


1, R = CH_3, R_1 = H
3, R = H, R_1 = CH_3
2

Fig. 1. Structures of compounds **1–3**.



1 **2**

Fig. 2. HMBC correlations of **1** and **2**.

and 58.7 (C-12), in addition to the IR absorption at 891 cm^{-1}, indicated the presence of an epoxide ring in **1**. The oxymethine proton at δ_H = 4.65 (dd, J = 9.4 and 6.9 Hz) was assigned to an α proton in the C-3 position bonded to an acyl group. The key HMBC cross-peaks between H-9/C-5, C-11, C-25 and C-26 (Fig. 2) established an epoxide ring between C-11 and C-12, while the cross-peaks between H_3-27/C-12, C-14 and C-18 indicated a double bond at C-14(15). Additional HMBC cross-peaks between H-18/C-12, C-20, C-27, C-28 and C-29, and between H-19/C-30, helped locate two secondary methyl groups at C-19 and C-20, respectively. On the basis of the above data, it was deduced that **1** possesses a D-friedours-11α,12α-epoxy-14-enyl skeleton, as previously reported for marsformoxide A, a constituent of *Marsdenia formosana* (Ito and Lai, 1979, 1978) and a synthetic product from α-amyrin (Agata et al., 1965). The connectivity between the *O*-cinnamoyl moiety and C-3 was evidenced by the HMBC cross-peaks between H-3/C-23, C-24 and C-9′. Compound **1** could therefore be identified as 3β-*O*-D-friedours-11α,12α-epoxy-14-enyl cinnamate.

Compound **2** was obtained as a colourless solid, m.p. = 184–185 °C, with a molecular formula of $C_{38}H_{54}O_3$ based on its HRESIMS (found $[M + Na]^+$ = 581.3942, calcd for $C_{38}H_{54}O_3Na$: 581.3957). The ^{13}C NMR spectrum exhibited 36 carbon resonances comprised of seven methyls, ten methylenes, 11 methines (including one oxymethine, two olefinic and three aromatic), as well as two carbonyls, and six quaternary carbons (including one aromatic). Based on the ^1H NMR spectrum, which showed resonances corresponding to a cinnamoyl group, as in **1** (Table 1), it could be deduced that the core triterpenoid skeleton of **2** has an empirical formula of $C_{29}H_{47}O_2$. The ^1H NMR spectrum (Table 1) showed resonance patterns similar to those of lupeol cinnamate, which was also isolated in this study, with a doublet of triplets at δ_H = 2.57 (J = 10.7 and 6.1 Hz) assigned to H-19, although the resonance appeared somewhat more downfield than the H-19 of lupeol cinnamate (at δ_H = 2.38), but with a singlet at δ_H = 2.12 corresponding to an acetyl group, and the absence of NMR resonances corresponding to a terminal double bond ca. δ_H = 4.69 and 4.58, and δ_C = 151.0 and 109.3 (Akihisa et at., 2010). The important HMBC cross-peak between H-19 and a carbonyl carbon resonance at δ_C = 212.3 led to the placement of a carbonyl functional group at C-20, and thus established a 29-*nor*-20-oxolupane structure in **2** (Fig. 2). The HMBC cross-peak between H-3/C-23, C-24 and C-9′ indicated a bond between C-3 and the *O*-cinnamoyl moiety. Compound **2** was finally elucidated as 3β-*O*-29-*nor*-20-oxolupanyl cinnamate.

Compound **3**, 3β-*O*-D-friedoolean-11α,12α-epoxy-14-enyl cinnamate, was reported to be previously isolated from *Gymnema alternifolium* (Lai et al., 1987), but no ^1H and ^{13}C NMR spectroscopic data were available, and these data were therefore included in Table 1.

In the present study, several triterpenoid cinnamate esters, together with an acetate and a palmitate ester, have been isolated, and some of these compounds, particularly lupeol cinnamate, have been reported to exhibit significant anti-inflammatory activity (Akihisa et al., 2010; de Miranda et al., 2000). The anti-inflammatory properties of this plant could thus be due partly to this compound. D-friedoolean-11α,12α-epoxy-14-enyl ester and/or D-friedours-11α,12α-epoxy-14-enyl ester are present in *Hoya kerrii*, *Gymnema acuminatum* (Lai et al., 1987), *Marsdenia formosana*, which was formerly assigned as an Asclepiadaceae plant, (Ito and Lai, 1979, 1978) and *Ecdysanthera rosea* (El-Kashef et al., 2015; Huang et al., 1990), all of which are plants in the Apocynaceae family, and could be of taxonomic importance.

3. Experimental

3.1. General experimental procedures

Melting points were measured using an electrothermal melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP 1020 polarimeter. The IR spectra were obtained on a Perkin-Elmer 1760x FT-IR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded with Bruker AVANCE 400 MHz and Bruker AVANCE III HD 400 MHz NMR spectrometers. Chemical shifts are referenced to the residual solvent signals ($CDCl_3$: δ_H = 7.24 and δ_C = 77.0 ppm). HRESIMS were recorded on a Bruker DaltonicsmicroTOF mass spectrometer.