Natural Product Research
Vol. 26, No. 8, April 2012, 749–755

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Long-chain alkanoic acid esters of lupeol from *Dorstenia harmsiana* Engl. (Moraceae)

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(*Received 20 September 2010; final version received 30 December 2010*)

In addition to lupeol (**1a**), three long-chain alkanoic acid esters of lupeol, in which two were new, were isolated from the hexane and ethyl acetate twigs extract of *Dorstenia harmsiana* Engl. (Moraceae). The structures of the new compounds were elucidated on the basis of 1D and 2D NMR experiments. Some isolated compounds were evaluated for their antimicrobial activities. The lupeol and its three long-chain alkanoic acid esters showed antifungal and antibacterial activities.

Keywords: *Dorstenia harmsiana*; Moraceae; lupeol; antimicrobial

1. Introduction

There are about 170 species of the genus *Dorstenia* worldwide (Mabberly, 1987). It is largely made up of undergrowth and herbaceous perennials with succulent and scrambling rhizomes (Berg, Human, & Weerdenburg, 1989). In many parts of Africa, *Dorstenia* species are commonly used for the treatment of an array of human disorders, including arthritis, rheumatism, gout, stomach disorders, cough, headache and skin diseases (Omisore et al., 2004).

To the best of our knowledge, no previous phytochemical study has been reported on *Dorstenia harmsiana* Engl. so far. However, the widespread use of *Dorstenia* in indigenous medicine for different ailments, as well as the antimicrobial activity exhibited by this genus (Mbaveng et al., 2008), justified further attempts to isolate and identify active compounds. This article deals with the isolation and the structural elucidation of two new long-chain alkanoic acid esters of lupeol, and their biological activities.

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ISSN 1478–6419 print/ISSN 1478–6427 online

http://dx.doi.org/10.1080/14786419.2010.551769
http://www.tandfonline.com

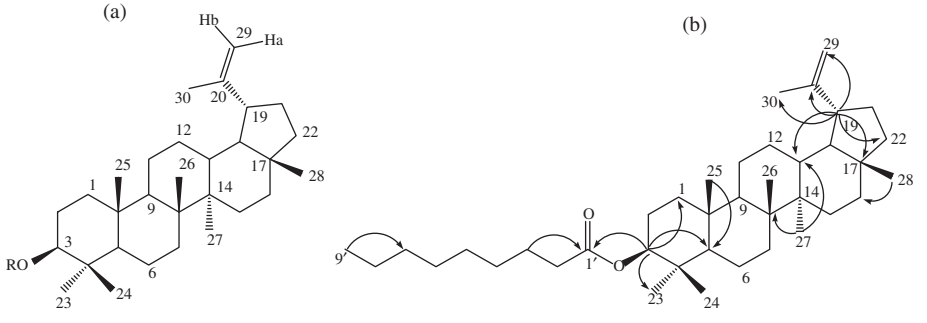
2. Results and discussion

The powdered twig of *D. harmsiana* was successively extracted with *n*-hexane (extract A) and ethyl acetate (extract B) in soxhlet apparatus. The extracts were submitted to repeated column chromatography and preparative TLC to afford fatty acid, lupeol (**1a**) and three long-chain alkanoic acid esters of lupeol (**1b–1d**) in which two were isolated and described here for the first time. The ^{1}H and ^{13}C NMR as well as the MS of the known compounds were consistent with those reported in the literature.

Compound **1b** was obtained as a white powder from extract A. The (+)-HR-ESI mass spectrum exhibited a pseudo-molecular ion of compound **1b** at m/z 567.5138 $[M+H]^+$, corresponding to a molecular formula $C_{39}H_{67}O_2$. The IR spectrum showed an ester signals at ν 1713 (C=O) and 1241 (C–O) cm^{-1}. Three absorption bands at ν 2930, 1633 and 878 cm^{-1} indicated a vinylidene group ($=CH_2$), characteristic of lup-20(29)-ene (Roitman & Jurd, 1978).

The ^{1}H NMR data indicated that compound **1b** (Figure 1) is a pentacyclic triterpenoid (Mbaze et al., 2007) with six methyl groups between δ 0.68 and 1.15 (s, 3H each), an olefinic methyl at δ 1.63 (s, 3H), a methylene at δ 2.18 (t, 2H, $J = 6.7$ Hz), six methine and 16 methylene groups between δ 1.12 and 1.90. A methine signal at δ 2.28 (ddd, $J = 11.8, 9.7, 4.7$ Hz) was attributed to the proton at C-19 position and one proton signal at δ 4.41 (dd, 1H, $J = 4.1, 10.6$ Hz) down field due to the nonanoxy moiety was attributed to the proton at C-3 position, and its characteristic of an equatorial (β) orientation. Two olefinic signals at δ 4.46 (brd, 1H, $J = 1.1$ Hz, Hb-29) and 4.62 (brd, 1H, $J = 1.1$ Hz, Ha-29) indicated an exomethylene group.

The ^{13}C NMR and DEPT spectra (Section 3) of compound **1b** revealed the presence of 39 carbon atoms, which were in accordance with the HR-ESI-MS and the proton data. The carbonyl was found at δ 174.0. The carbon atoms at δ 151.3 and 109.7 are characteristic for the carbons 20 and 29 of lup-20(29)-ene (Mbaze et al., 2007), respectively. These data indicated that compound **1b** might be a lupan-type triterpene. The fragment at m/z 189 supported the presence of lup-20(29)-ene (Kumar, Muthukuda, & Wazeer, 1985).



1a: R=H; **1b**: R=$C_8H_{17}CO$;

1c: R=$C_{10}H_{21}CO$;**1d**: R=$C_{15}H_{31}CO$

Figure 1. (**a**) Structures of compounds **1a–1d.** (**b**) Selected HMBC correlation in compound **1b**. Respective correlations were found also in compound **1c** and **1d**.

In the HMBC spectrum (Figure 1), correlations between the H-3 signal and carbons 1, 2, 4, 5, 23, 24 and 1′, and the correlation of H-19 with C-17, 18, 20, 21, 22, 29 and 30 as well as the similarity with the shifts of lupeol (**1a**) indicated that compound **1b** has a long-chain ester moiety at the position C-3 (Figure 1) (Razdan, Kachroo, Qurishi, Kalla, & Waight, 1996). Thus compound **1b** was characterised as lup-20(29)-en-3-nonanoate, which is described here for the first time.

1D and 2D NMR spectra of compounds **1b** and **1c** were super-imposable, suggesting that they belonged to a homologous series of lupeol with a long side chain at C-3 position. The only differences between compounds **1b** and **1c** were in their mass spectra and physical properties (Section 3). The positive HR-ESI mass spectrum of **1c** exhibited a pseudo-molecular ion at m/z 595.5451 $[M+H]^+$, $C_{41}H_{71}O_2$ (**1c**), thus compounds **1c** was characterised as lup-20(29)-en-3-undecanoate. Compound **1d** identified as lup-20(29)-en-3-hexadecanoate was also isolated in *Koelpinia linearis* (Compositeae) (Razdan et al., 1996).

The chemical constituents of the genus *Dorstenia* have been reported to be coumarins/furanocoumarins (Abegaz et al., 2004; Caceres et al., 2001; Franke, Porzel, Masaoud, Adam, & Schmidt, 2001; Rojas-Lima, Santillan, DomõÂnguez, & GutieÂrrez, 1999; Swain, Quirke, Winkle, & Downum, 1991), flavonoids (Abegaz et al., 2002, 2004; Ngadjui, Dongo, Abegaz, Fotso, & Tamboue, 2002; Ngadjui, Dongo, Tamboue, Kouam, & Abegaz, 1999; Ngadjui, Kapche, Tamboue, Abegaz, & Connolly, 1999; Ngadjui, Kouam, Dongo, Kapche, & Abegaz, 2000; Ngadjui, Tabopda, et al., 1999; Ngadjui et al., 2005; Ngameni, Ngadui, Folefoc, Watchueng, & Abegaz, 2004; Ngameni et al., 2006) and triterpenoids (Uchiyama, Hara, Makino, & Fujimoto, 2002; Vouffo et al., 2008). This is the first time that long-chain alkanoic acid ester has been isolated from *Dorstenia* genus.

Hydrolysis of **1b** and **1c** gave **1a**, nonanoic acid and undecanoic acid (Section 3). The structures of nonanoic acid and undecanoic acid were confirmed by HR-EI-MS and ^{1}H NMR spectra.

Since some of these genus are known to show some antimicrobial activity (Mbaveng et al., 2008), we investigated whether isolated compounds (**1a–1d)** would also exhibit antimicrobial activity. The antifungal and antibacterial activities of **1a–1d** were determined using the agar diffusion method with 8 mm paper disks loaded with 40 μg of each compound while nystatin (*Staphylococcus aureus* = 12, *Bacillus subtilis* = 16, *Streptomyces viridochromogenes* (Tü 57) = 14, *Escherichia coli* = 14) was used as the reference. Compounds **1a**, **1b**, **1c** and **1d** showed activities against *Staphylococcus aureus* (9, 12, 12, and 13 mm inhibition diameter), *Bacillus subtilis* (9, 10, 10 and 10 mm), *Streptomyces viridochromogenes* (Tü 57) (11, 12, 12 and 13 mm) and *Escherichia coli* (12, 11, 12 and 12 mm), respectively.

3. Experimental section

3.1. *General*

Melting point is uncorrected and was obtained with a micro melting point apparatus (Yanaco, Tokyo-Japan). Optical rotation values were measured with a Horiba SEPA-300 polarimeter, and IR spectra were recorded with JASCO J-20A spectrophotometer. The ^{1}H and ^{13}C NMR spectra were acquired with a Jeol EX-400 spectrometer. Chemical shifts are given on a δ (ppm) scale with TMS as an

internal standard. Mass spectra were obtained with a Jeol JMS-700 instrument. Column chromatography was conducted on silica gel 60 (Kanto Chemical Co., Inc., Japan), Sephadex LH-20 (Pharmacia, Sweden) and ODS (Fuji Silysia, Japan). TLC analysis was carried out by using precoated silica gel plates (Merck), and the spots were detected by spraying with H_2SO_4/10% vanillin and then heating. Flash chromatography was carried out on silica gel (230–400 mesh). R_f values were measured on PolyGram SIL G/UV254 (Macherey-Nagel & Co.).

3.2. *Extraction and isolation*

The twigs of *D. harmsiana* were collected in July 2008 at Kribi, South Cameroon. A specimen has been deposited in the National Herbarium, Yaoundé, Cameroon (Ref. No. 21642/SRF).

Powdered twigs (500 g) of *D. harmsiana* were successively extracted with *n*-hexane and ethyl acetate in soxhlet apparatus. The solvent was removed under reduced pressure to yield 12 and 9 g extract of *n*-hexane (extract A) and ethyl acetate (extract B), respectively.

Extract A (12 g) was chromatographed over silica gel using hexane–acetone of increasing polarity, which yielded two main fractions. Fraction I (2.2 g) was purified by a small column chromatography using Sephadex LH-20 and $CHCl_3$: MeOH (7 : 2) as eluent, followed by preparative TLC to afford lupeol (**1a**, 85.1 mg) (Poumale, 2007) and lup-20(29)-en-3-nonanoate (**1b**, 28.0 mg). Fraction II (0.9 g) was purified by a silica gel column chromatography eluted with hexane:ethyl acetate (4 : 1) to yield lup-20(29)-en-3-undecanoate (**1c**, 24.3 mg).

Extract B (9.0 g) was passed through a Sephadex LH-20 column and subjected to silica gel column chromatography and preparative TLC to afford lupeol (**1a**, 14.9 mg) and lup-20(29)-en-3-hexadecanoate (**1d**, 31.0 mg) (Razdan et al., 1996).

3.2.1. *Lup-20(29)-en-3-nonanoate (**1b**)*

White powder; m.p.: 78–79°C; $R_f = 0.53$ ($CHCl_3$); $[\alpha]_D + 26.7°$ (*c* 0.1, $CHCl_3$); IR (film) ν_{max}: 2947, 2873, 1724, 1641, 1451, 1375, 1247, 1183, 1040, 1020, 975, 888 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.62 (brd, 1H, $J = 1.1$ Hz, Ha-29), 4.46 (brd, 1H, $J = 1.1$ Hz, Hb-29), 4.41 (dd, 1H, $J = 10.6$, 4.1 Hz, H-3), 2.28 (ddd, 1H, $J = 11.8$, 9.7, 4.7 Hz, H-19), 2.18 (t, 2H, $J = 6.7$ Hz, H-2'), 1.63 (s, 3H, H-30), 1.11-1.90 (overlapping multiplets, 39H, H-1, H-2, H-5, H-6, H-7, H-9, H-11, H-12, H-13, H-15, H-16, H-18, H-21, H-22, H-3′, H-4′, H-5′, H-6′; H-7′, H-8′, H-9′), 1.12 (s, 3H, H-23), 0.90 (s, 3H, H-24), 0.80 (s, 3H, H-27), 0.78 (s, 3H, H-26), 0.77 (s, 3H, H-25), 0.69 (s, 3H, H-28); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.0 (s, C-1′), 151.3 (s, C-20), 109.7 (t, C-29), 80.9 (d, C-3), 55.7 (d, C-5), 50.6 (d, C-9), 48.6 (d, C-18), 48.3 (d, C-19), 43.3 (s, C-17), 43.1 (s, C-14), 41.2 (s, C-8), 40.3 (t, C-22), 38.7 (t, C-1) 38.7 (d, C-13), 38.6 (t, C-15), 38.1 (s, C-4), 37.4 (s, C-10), 35.9 (t, C-16), 35.2 (t, C-7), 34.5 (t, C-2′), 32.2 (t, C-21), 30.1-27.7 (overlapping triplets, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′), 25.5 (t, C-12), 25.4 (t, C-2), 21.2 (t, C-11), 24.1 (q, C-23), 18.5 (t, C-6), 19.6 (q, C-30), 18.2 (q, C-28), 16.9 (q, C-25), 16.4 (q, C-26), 16.3 (q, C-27), 14.8 (q, C-24), 14.4 (q, C-9′); EI-MS (70 eV): *m*/*z* (%) 566 (M^+, 9), 538(16), 530 (8), 513 (12), 460 (6), 425 ([M − $C_8H_{17}CO]^+$,18), 409 (90), 407 (9), 367 (8), 365 (10), 307 (42), 289 (34), 229 (12), 203

(40), 189 (21), 154 (100) 143 (90), 136 (94), 107 (7), 81 (10), 55 (13), 43 (40); (+)-HR-ESI-MS: m/z 567.5138 ($[M+H]^+$, Calcd 567.5141 for $C_{39}H_{67}O_2$).

3.2.2. *Lup-20(29)-en-3-undecanoate (**1c**)*

White powder; m.p. = 83–84°C; $R_f = 0.61$ ($CHCl_3$); $[\alpha]_D + 38.6°$ (c 0.5, $CHCl_3$); IR (film) (See **1b);** ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.62 (brd, 1H, $J = 1.1$ Hz, Ha-29), 4.46 (brd, 1H, $J = 1.1$ Hz, Hb-29), 4.41 (dd, 1H, $J = 10.6$, 4.1 Hz, H-3), 2.28 (ddd, 1H, $J = 11.8$, 9.7, 4.7 Hz, H-19), 2.18 (t, 2H, $J = 6.7$ Hz, H-2′), 1.63 (s, 3H, H-30), 1.11-1.90 (overlapping multiplets, 43H, H-1, H-2, H-5, H-6, H-7, H-9, H-11, H-12, H-13, H-15, H-16, H-18, H-21, H-22, H-3′, H-4′, H-5′, H-6′; H-7′, H-8′, H-9′, H-10′, C-11′), 1.12 (s, 3H, H-23), 0.90 (s, 3H, H-24), 0.80 (s, 3H, H-27), 0.78 (s, 3H, H-26), 0.77 (s, 3H, H-25), 0.69 (s, 3H, H-28); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.0 (s, C-1′), 151.3 (s, C-20), 109.7 (t, C-29), 80.9 (d, C-3), 55.7 (d, C-5), 50.6 (d, C-9), 48.6 (d, C-18), 48.3 (d, C-19), 43.3 (s, C-17), 43.1 (s, C-14), 41.2 (s, C-8), 40.3 (t, C-22), 38.7 (t, C-1) 38.7 (d, C-13), 38.6 (t, C-15), 38.1 (s, C-4), 37.4 (s, C-10), 35.9 (t, C-16), 35.2 (t, C-7), 34.5 (t, C-2′), 32.2 (t, C-21), 30.1-27.7 (overlapping triplets, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-9′, C-10′), 25.5 (t, C-12), 25.4 (t, C-2), 21.2 (t, C-11), 24.1 (q, C-23), 18.5 (t, C-6), 19.6 (q, C-30), 18.2 (q, C-28), 16.9 (q, C-25), 16.4 (q, C-26), 16.3 (q, C-27), 14.8 (q, C-24), 14.4 (q, C-11′); EI-MS (70 eV): m/z (%) 594 (M^+, 10); (+)-HR-ESI-MS: m/z 595.5451 ($[M+H]^+$, calcd 595.5454 for $C_{41}H_{71}O_2$).

3.2.3. *Hydrolysis of **1b** and **1c***

Compounds **1b** and **1c** (4.0 mg each) were dissolved separately in MeOH (1 mL)/distilled H_2O (1 mL). Then 7% HCl (1 mL) solution was added and the solution was refluxed at 39°C for 4 h. Usual work up gave lupeol **1a**, nonanoic acid (1.7 mg) and undecanoic acid (1.3 mg).

Nonanoic acid. Amorphous solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (t, 2H), 1.20 (brs, 12H), 0.88 (t, 3H); HR-EI-MS m/z 158.1306 (Calcd 158.1307 for $C_9H_{18}O_2$).

Undecanoic acid. Amorphous solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (t, 2H), 1.20 (brs, 16H), 0.88 (t, 3H); HR-EI-MS m/z 186.1618 (Calcd 186.1620 for $C_{11}H_{22}O_2$).

3.3. *Antimicrobial assay*

Agar diffusion tests were performed in the usual manner (Poumale et al., 2008) with *B. subtilis* and *E. coli* (on peptone agar), *S. aureus* (Bacto nutrient broth), *S. viridochromogenes* (M Test agar), the fungi *Mucor miehei* and *Candida albicans* (Sabouraud agar), and three microalgae (*Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*).

Compounds were dissolved in an azeotrope chloroform:MeOH (87 : 13) and 40 μg pro paper disks (Ø8 mm) were impregnated with each using a 100 μL syringe, dried for 1 h under sterile conditions and placed on the pre-made agar test plates. Bacteria and fungi plates were kept in an incubator at 37°C for 12 h and micro algae plates were kept for 3 days at room temperature in a day light incubator. The diameter of inhibition zones was measured.

4. Conclusion

Lupeol and three long-chain alkanoic acid esters of lupeol were isolated form the twigs extract of *D. harmsiana*. Hydrolysis of the new compounds was carried out for the confirmation of their structures. The isolated compounds showed some antimicrobial activity at 40 μg pro paper disks. Flavonoids and coumarins which are common in this genus were not isolated.

Acknowledgements

The authors are grateful to the Japan Society for the Promotion of Science (JSPS) for the fellowship (No. P08430) awarded to Dr H.M.P. Poumale at the University of Yamagata, Japan.

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