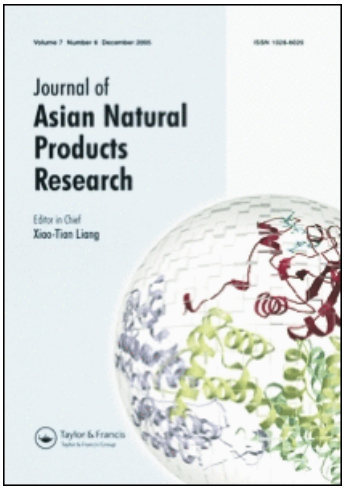

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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XIAO-HUA Xu[a]; NIAN-YUN Yang[a]; SHI-HUI Qian[b]; NING Xie[a]; JING-AO Duan[b]
[a] Department of Phytochemistry, China Pharmaceutical University, Nanjing, China [b] Jiangsu Institute of Traditional Chinese Medicine, Nanjing, China

To cite this Article Xu, XIAO-HUA , Yang, NIAN-YUN , Qian, SHI-HUI , Xie, NING and Duan, JING-AO(2008) 'Dammarane triterpenes from *Ligustrum lucidum*', Journal of Asian Natural Products Research, 10: 1, 33 — 37
To link to this Article: DOI: 10.1080/10286020701273833
URL: http://dx.doi.org/10.1080/10286020701273833

Journal of Asian Natural Products Research, Vol. 10, No. 1, January 2008, 33–37

Dammarane triterpenes from *Ligustrum lucidum*

XIAO-HUA XU†‡, NIAN-YUN YANG†‡, SHI-HUI QIAN‡*, NING XIE† and JING-AO DUAN‡

†Department of Phytochemistry, China Pharmaceutical University, Nanjing 210009, China
‡Jiangsu Institute of Traditional Chinese Medicine, Nanjing 210028, China

(Received 28 August 2006; revised 14 December 2006; in final form 22 December 2006)

Two new dammarane triterpenes 3β-acetyl-20*S*,24*R*-dammarane-25-ene-24-hydroperoxy-20-ol (**1**), 20*S*,24*R*-dammarane-25-ene-24-hydroperoxy-3β,20-diol (**2**), as well as three known dammarane triterpenes, 3β-acetyl-20*S*,25-epoxydammarane-24α-ol (**3**), 20*S*,25-epoxydammarane-3β,24α-diol (**4**), 20*S*-dammarane-23-ene-3β,20,25-triol (**5**) were isolated from the fruits of *Ligustrum lucidum*. Compounds **3**–**5** were isolated from the fruits of *L. lucidum* for the first time. Their structures were elucidated by spectroscopic methods.

Keywords: *Ligusturm lucidum*; Oleaceae; Dammarane; Triterpenes

1. Introduction

The fruits of *Ligustrum lucidum* Ait. (Oleaceae) are known as Nuzhenzi which are commonly used for their tonic effects in Chinese medicine [1]. Previous studies had found volatile components, triterpenes, flavonoids, secoiridoid glucosides, and phenolic compounds from this plant [2–4]. In our detailed survey of the fruits of *L. lucidum*, two new dammarane triterpenes, **1** and **2**, had been isolated as well as three known compounds **3**–**5** [5–7] (figure 1). The structures of those compounds were identified by means of one and two dimensional NMR spectroscopic techniques, including HSQC, HMBC, and NOESY.

2. Results and discussion

All compounds (**1**–**5**) were obtained from the petroleum ether soluble fraction of 80% ethanol extract from the fruits of *L. lucidum* by column chromatography on silica gel as described in the experiment. Identifications of **3**–**5** were achieved by comparison with previously reported spectroscopic data.

*Corresponding author. Email: njqsh2005@126.com

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2008 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020701273833

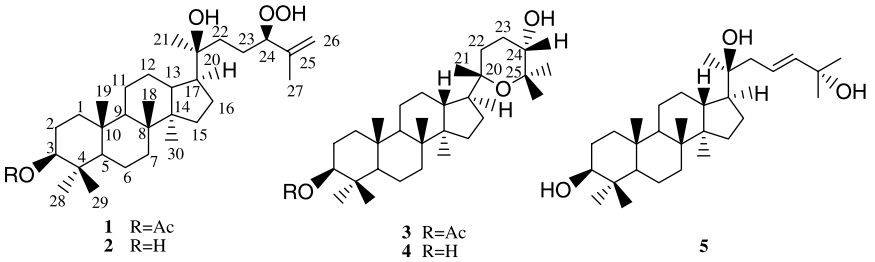

Figure 1. The structures of **1**–**5**.

Compound **1** showed a quasi-molecular ion peak $[M + Na]^+$ at *m/z* 541. HRTOF-MS assigned the molecular formula of $C_{32}H_{54}O_5$. The ^1H NMR spectrum of **1** (table 2) revealed seven methyl groups (δ 2 × 0.85, 2 × 0.87, 0.95, 1.12, 1.75), the ^{13}C NMR spectrum of **1** (table 1) showed 32 carbon signals, which typically represent the dammarane skeleton [7], and the carbon signals at δ 21.2, 171.0 revealed the presence of an acetyl carbonyl group.

In the HMQC spectrum, the oxymethine proton (δ 4.48) was attached to a carbon at δ 80.9, suggesting the corresponding carbon was esterified. The large coupling constants (10.5 and 5.5 Hz) of this proton demonstrated the equatorial β-orientation of the acetyl group [5]. Compound **1** was shown to possess a hydroperoxyl group by its positive response to the ferrous thiocyanate reagent [8,9]. The ^{13}C NMR signals of **1** were similar to those of compound **3** except

Table 1. ^{13}C NMR spectral data of compounds **1**–**5** in $CDCl_3$ (125 MHz).

Carbon	***1***	***2***	***3***	***4***	***5***
1	38.7	39.0	38.7	39.0	38.9
2	23.7	24.9	23.7	24.8	24.8
3	80.9	78.9	80.9	78.9	78.9
4	37.9	39.0	37.9	39.1	39.0
5	55.9	55.9	55.9	55.9	55.9
6	18.1	18.3	18.2	18.3	18.2
7	35.2	35.2	35.7	35.3	35.2
8	40.4	40.4	40.4	40.4	40.4
9	50.6	50.6	50.7	50.8	50.6
10	37.1	37.1	37.1	37.2	37.1
11	21.5	21.5	21.6	21.6	21.5
12	27.4	27.4	27.3	27.4	27.4
13	42.4	42.4	42.9	42.9	42.4
14	50.3	50.3	50.0	50.0	50.3
15	31.2	31.2	31.5	31.5	31.1
16	25.2	25.3	25.7	25.7	24.8
17	49.7	49.7	49.6	49.6	49.9
18	15.4	15.3	15.4	15.4	15.3
19	16.4	16.4	16.4	16.4	16.4
20	75.1	75.1	86.4	86.4	75.1
21	24.7	24.8	23.5	23.5	25.6
22	36.3	36.3	35.2	35.7	43.4
23	24.9	24.9	26.1	26.1	122.3
24	89.7	89.8	83.3	83.3	142.0
25	143.7	143.6	71.4	71.4	71.2
26	114.1	114.2	24.3	24.3	29.7
27	17.5	17.5	27.4	27.4	29.8
28	16.2	28.0	16.3	28.0	28.0
29	27.9	15.5	27.9	15.3	15.5
30	16.4	16.2	16.4	16.2	16.2
$\underline{C}H_3CO$	21.2		21.3		
$\overline{C}H_3\underline{C}O$	171.0		170.9		

for the signals due to the side-chain part (C-21—C-27). The structure of the side-chain part were determined by HMQC, HMBC, and NOE experiments. A singlet of methylene protons at δ 5.02 (2H, s) suggested the presence of methylene moiety in the side chain. The carbon signal at δ 89.7 was assigned to C-24, because H-24 was observed as triplet-like signal ($J = 12.6$ Hz) due to coupling with H-23 [9], in the HMBC spectrum, the methine proton signal at δ 4.28 (1H, t, $J = 12.6$ Hz) showed correlations with C-22 (δ 36.3), C-23 (δ 24.9), C-25 (δ 143.7), C-26 (δ 114.1) and C-27 (δ 17.5), so this proton signal was assigned to H-24. These findings proved the hydroperoxyl group at C-24. Furthermore other correlation were also observed from the following protons and carbons: H-21 (δ 1.12)/C-20, C-22; H-27 (δ 1.75)/C-24, C-25, C-26; H-22 (δ 1.48, 1.53)/C-20, C-21, C-23, C-24; H-23 (δ 1.47,1.71)/C-20, C-22, C-24, C-25; H-26 (δ 5.02)/C-25, C-24, C-27 (figure 2), confirmed the structure of the side chain in **1**.

The relative stereochemistry of four-ring systems was clarified by the NOESY spectrum. The configuration of C-17 was assigned as S by the correlation between H-17 (δ 1.74) and H-30 (δ 0.87) in NOESY spectrum. Correlations between H-21 and H-16, 17 were not detected; however, strong interactions between the H-21 signal at δ 1.12 and H-22, H-23 were observed in NOESY spectrum as shown in figure 2, which led to the assignment of C-20 configuration as S [10,11]. Correlations between H-24 at δ 4.28 and H-22, H-23, H-26, and H-27 were observed in NOESY spectrum as shown in figure 2, which led to the assignment of C-24 configuration as R. Thus the structure of **1** was determined as 3β-acetyl-20*S*,24*R*-dammarane-25-ene-24-hydroperoxy-20-ol.

Compound **2** showed a quasi-molecular ion peak $[M + Na]^+$ at *m*/*z* 499. HRTOF-MS assigned the molecular formula $C_{30}H_{52}O_4$. Compared with **1**, the ^{1}H NMR and ^{13}C NMR spectra of compound **2** (tables 1 and 2) were almost identical to those of **1**, except for the absence of an acetyl group in **2**. In ^{1}H NMR spectrum, signal of H-3 (δ 3.20, dd, $J = 10.5$, 5.5 Hz) shifted towards upfield, compared with that of **1** (δ 4.48, dd, $J = 10.5$, 5.5 Hz), suggesting the corresponding carbon was not esterified. In the HMBC spectrum, H-3 (δ 3.20) correlated with C-1 (δ 39.0), C-2 (δ 24.9), C-4 (δ 39.0), C-28 (δ 28.0), C-29 (δ 15.5). Thus the structure of **2** was deduced as 20*S*,24*R*-dammarane-25-ene-24-hydroperoxy-3β,20-diol.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT4A micromelting apparatus and are uncorrected. IR spectra were measured on a Perkin–Elmer 8900 FT-IR instrument as KBr disks. 1D and 2D NMR spectra were recorded in $CDCl_3$ on a Varian-500 spectrometer. HRTOF-MS and SCI-MS

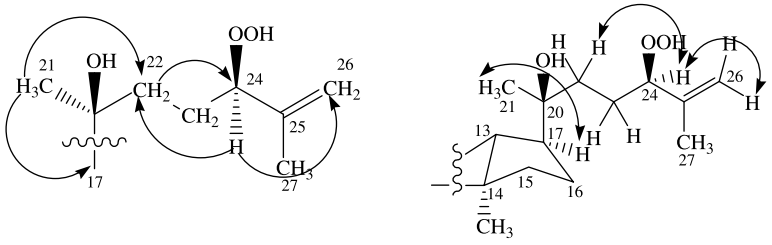

Figure 2. Key HMBC and NOESY correlations of **1**.

Table 2. ^{1}H NMR spectral data of compounds **1**–**5** in $CDCl_3$ (500 MHz).

H	***1*** *J (Hz)*	***2*** *J (Hz)*	***3*** *J (Hz)*	***4*** *J (Hz)*	***5*** *J (Hz)*
3	4.48 dd 10.5, 5.5	3.20 dd 10.5, 5.5	4.47 dd 10.5, 5.5	3.19 dd 10.5 5.5	3.19 dd 10.5, 5.5
18	0.95 s	0.96 s	0.95 s	0.96 s	0.96 s
19	0.85 s	0.85 s	0.84 s	0.84 s	0.86 s
21	1.12 s	1.11 s	1.05 m	1.08 m	1.12 s
22	1.48 m	1.47 m	1.57 m	1.58 m	2.19 dd 7.0, 5.0
	1.53 m	1.53 m	1.68 m	1.67 m	
23	1.47 m	1.46 m	1.74 m	1.73 m	5.70 m
	1.71 m	1.70 m	1.82 m	1.83 m	
24	4.28 t 12.6	4.28 t 12.6	3.72 t 14.4	3.73 t 14.4	5.70 m
26	5.02 s	5.02 s	1.11 s	1.12 s	1.32 s
27	1.75 s	1.73 s	1.12 s	1.13 s	1.32 s
28	0.87 s	0.99 s	0.86 s	0.96 s	0.97 s
29	0.85 s	0.77 s	0.84 s	0.77 s	0.77 s
30	0.87 s	0.87 s	0.86 s	0.86 s	0.84 s
CO	2.01 s		2.04 s		
OOH	8.11 s	8.12 s			

spectra were performed on Bruker APEX-α mass spectrometer and Micromass Qauttro micro mass spectrometer respectively. Column chromatography was carried out on silica gel (200–300 mesh Qingdao Haiyang Chemical Co. Ltd, China) and Sephadex LH-20 (Pharmacia Biotech).

3.2 Plant material

The fruits of *Ligustrum lucidum* were collected from Nanjing city, Jiangsu Province, China in November 2004 and identified by Researcher Qian. A voucher specimen (No. S-07-00020) is deposited in the Jiangsu Institute of Traditional Chinese Medicine.

3.3 Extraction and isolation

Air-dried fruits (8 kg) were extracted with 80% ethanol (2 × 50 L) for 2 h under reflux, and the combined extracts were concentrated *in vacuo*. The obtained extract (1.835 kg) was then suspended in H_2O and extracted successively with petroleum ether, EtOAc, *n*-butanol saturated with H_2O to give the respective extracts after solvent removal. The petroleum ether soluble portion (231 g) was eluted with petroleum ether/EtOAc by column chromatography on silica gel. Based on TLC characteristics, six major fractions I–VI were made. Fraction α (19.4 g) was further isolated by repeated column chromatography on silica gel with petroleum ether/EtOAc (10:1) to afford **3** (56 mg) and the following four subfractions αa–d. Subfraction αb was purified by silica gel with petroleum ether/EtOAc (96:4) as eluent, and afforded 1 (30 mg). Fraction β (13.1 g) was fractionated by repeated column chromatography on silica gel with petroleum ether/EtOAc (96:4) to give four subfractions βa–d. Compounds **2** (13 mg) and **4** (20 mg) were obtained from subfractions βb and βc, respectively, with petroleum ether/EtOAc (93:7). Fraction βd was purified by Sephadex LH-20 with $CHCl_3$/MeOH (1:1), and **5** (6 mg) was obtained.

3.3.1 3β-Acetoxyl-20*S*,24*R*-dammarane-25-ene-24-hydroperoxy-20-ol (1). White powder, mp 220–222°C. HRTOF-MS *m/z* 541.3886 $[M + Na]^+$ (calcd for $C_{32}H_{54}O_5Na$, 541.3881); IR (KBr) (ν_{max} cm^{-1}): 3420, 1718, 1455, 1381, 1255, 1123, 1020, 900; ^{1}H NMR and ^{13}C NMR ($CDCl_3$) spectral data: see tables 1 and 2, respectively.

3.3.2 20*S*,24*R*-Dammarane-25-ene-24-hydroperoxy-3β,20-diol (2). White powder, mp 197–200°C. HRTOF-MS *m/z* 499.3742 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_4Na$, 499.3736); IR (KBr) (ν_{max} cm^{-1}): 3425, 1453, 1378, 1249, 1126, 1017, 920; ^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data: see tables 1 and 2, respectively.

3.3.3 3β-Acetoxyl-20*S*,25-epoxydammarane-24α-ol (3). White powder, mp 265–266°C. ESI-MS *m/z* 526.4 $[M + Na + H]^+$; IR (KBr) (ν_{max} cm^{-1}): 3472, 1723, 1449, 1383, 1261, 1123, 1020, 960; ^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data: see tables 1 and 2, respectively.

3.3.4 20*S*,25-Epoxydammarane-3β,24α-diol (4). White powder, mp 196–198°C. ESI-MS *m/z* 484.1 $[M + Na + H]^+$; IR (KBr) (ν_{max} cm^{-1}): 3467, 1451, 1377, 1246, 1119, 1022, 945; ^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data: see tables 1 and 2, respectively.

3.3.5 20*S*-Dammarane-23-ene-3β,20,25-triol (5). White powder, mp 107–109°C. ESI-MS *m/z* 484.2 $[M + Na + H]^+$; IR (KBr) (ν_{max} cm^{-1}): 3467, 1461, 1382, 1249, 1131, 1028, 936; ^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data: see tables 1 and 2, respectively.

3.4 Validation of the hydroperoxyl group

A solution of 0.5 mg of compound **1** in 1 ml anhydrous Et_2O was prepared, and 0.2 ml 0.1 mol/L fresh $FeSO_4$ solution was added, and acutely surged. The colour of solution changed from green to red.

Acknowledgements

The programme was supported by the Commonweal Foundation of Jiangsu Province (grant No: BM2004525).

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