A Hydroxylated Lupeol-Based Triterpenoid Ester Isolated from the *Scurrula parasitica* Parasitic on *Nerium indicum*

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(3*β*,7*β*)-7-Hydroxylup-20(29)-en-3-yl hexadecanoate (**1**), a new lupeol-based triterpenoid ester, along with sixteen known compounds, 7*β*,15*α*-dihydroxylup-20(29)-ene-3*β*-*O*-palmitate (**2**), lupeol palmitate (**3**), lupeol (**4**), 3-oxolup-20(29)-ene (**5**), ursolic acid (**6**), cycloeucalenol (**7**), stigmasterol (**8**), *β*-sitosterol (**9**), *β*-daucosterol (**10**), quercetin (**11**), quercetin 3-*O*-*α*-L-arabinoside (**12**), quercetin 3-*O*-*α*-L-rhamnoside (**13**), catechin (**14**), gitoxigenin 3-*O*-*α*-L-rhamnoside (**15**), gitoxigenin 3-*O*-*α*-D-glucoside (**16**), and digitoxigenin 3-*O*-*α*-L-rhamnoside (**17**), was isolated from the leaves of the Southern China mistletoe, *Scurrula parasitica* LINN parasitic on *Nerium indicum* MILL. Their structures were elucidated by spectroscopic analyses, including 2D-NMR techniques. Cytotoxic activities of compounds **1**–**7** and **11**–**17** were evaluated against three cancer cell lines, PANC-1, HL-60, and SGC-7901, revealing that compounds **4**, **6**, **11**, and **15**–**17** exhibited effective cytotoxicities, while others were inactive. A structure–activity relationship study of compounds **1**–**5** indicated that the 3-OH group in lupeol-based triterpenoids is essential for antitumor activity.

Introduction. – Throughout the world, mistletoes occur as semiparasitic evergreen plants which depend on their host trees for minerals and water only, but photosynthesize their carbohydrates by means of its green leaves [1]. There are a lot of species known worldwide [2], and scientific evidence has shown that their compositions or activities are dependent on the host tree, species, and harvesting period [3–5]. *Scurrula parasitica* L. (Loranthaceae) is one of the most common parasitc *Scurrula* plants in the austral area of China, and its leaves and stems have been used as cardiotonic, antioxidant, and antineoplastic agents [6]. As expected, these activities varied with the host trees and seasons [7]. The total flavonoid extract of *Scurrula parasitica* L. parasitic on four different host trees with mistletoe from *Nerium indicum* exhibited the highest anticancer activity [8]. Although some activities have been reported for the *Scurrula parasitica* parasitic on *Nerium indicum*, conclusive information regarding its chemical constituents is lacking. This study, therefore, involves the isolation and characterization of some of the triterpenoids, flavonoids, and cardiac glycosides from the MeOH extracts of the Southern China mistletoe epiphytic on *Nerium indicum*. To the best of our knowledge, this is the first attempt to isolate and characterize chemical constituents, **1**–**17** (*Fig. 1*) and their cytotoxicities from the *Scurrula parasitica* L. parasitic on *Nerium indicum* MILL.

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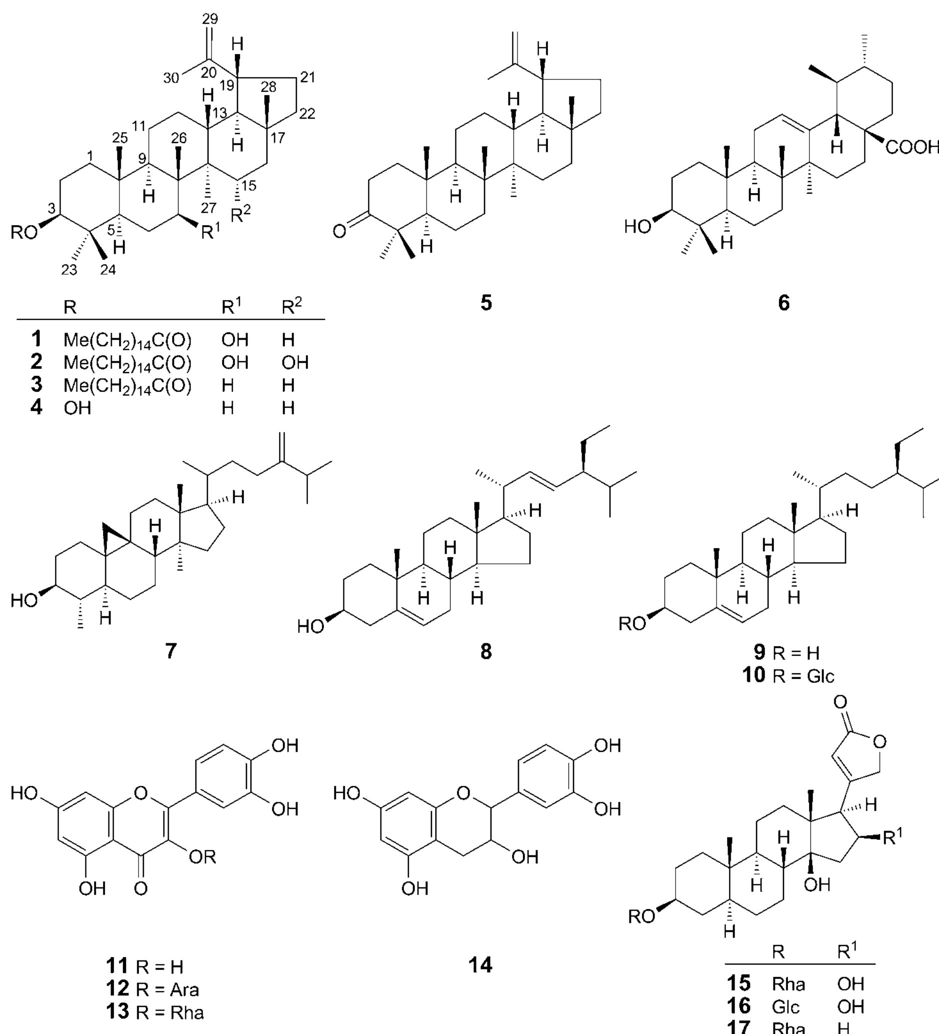

Fig. 1. *Compounds* **1**–**17**, *isolated from* Scurrula parasitica L. *parasitic on* Nerium indicum MILL

Results and Discussion. – Compound **1** was obtained as white amorphous powder. Its molecular formula was deduced as $C_{46}H_{80}O_3$ from high-resolution ESI mass spectra (*m*/*z* 703.6010 ($[M+Na]^+$; calc. 703.6005)). It gave a positive *Liebermann–Burchard* test for triterpenoids. The ^{1}H-NMR spectrum of **1** (*Table 1*) exhibited due to seven *singlet*s Me (δ(H) 0.79, 0.84, 0.85, 0.86, 1.06, 1.41, and 1.68) and a Me group *triplet-doublet* at δ(H) 0.88. The presence of a secondary OH group at C(7) in a lupeol nucleus was evidenced by the ^{1}H-NMR signal at δ(H) 3.82 (*dd*, $J = 10.8$, 4.8, 1 H). In addition, the CH group at δ(H) 2.37 (*dt*, $J = 7.8$, 5.6, 1 H) characteristic for a lupeol-type triterpene. Additionally, a terminal Me signal around δ(H) 0.88 and a strong CH_2 group H-atom signals around δ(H) 1.25 were indicative of a fatty-acid chain, which was

Table 1. *^{1}H- and ^{13}C-NMR* (600 and 150 MHz, resp.; in $CDCl_3$), *and HMBC Data of Compound* **1**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	HMBC(H → C)
1	0.93 – 0.96 (*m*), 1.66 – 1.68 (*m*)	38.2 (*t*)	C(2), C(10), C(25)
2	1.27 – 1.29 (*m*)	23.7 (*t*)	C(1), C(3)
3	4.47 (*dd*, J = 11.2, 4.4)	80.1 (*d*)	C(2), C(4), C(7), C(23), C(24), C(1′)
4		37.5 (*s*)	
5	0.86 – 0.88 (*m*)	52.3 (*d*)	C(4), C(6), C(10), C(23), C(24)
6	1.30 – 1.32 (*m*)	29.0 (*t*)	C(5), C(7), C(10)
7	3.82 (*dd*, J = 10.8, 4.8)	74.4 (*d*)	C(6), C(8), C(26)
8		44.2 (*s*)	
9	1.21 – 1.23 (*m*)	50.1 (*d*)	C(8), C(10), C(26)
10		37.0 (*s*)	
11	1.42 – 1.44 (*m*)	20.8 (*t*)	C(12)
12	1.60 – 1.61 (*m*)	25.1 (*t*)	C(11), C(13)
13	1.62 – 1.63 (*m*)	38.3 (*d*)	C(12), C(14)
14		42.6 (*s*)	
15	1.23 – 1.25 (*m*)	29.4 (*t*)	C(8), C(16), C(18), C(27)
16	1.37 – 1.39 (*m*)	35.9 (*t*)	C(15), C(17), C(28)
17		46.7 (*s*)	
18	1.36 – 1.37 (*m*)	48.1 (*d*)	C(13), C(19)
19	2.37 (*dt*, J = 7.8, 5.6)	47.1 (*d*)	C(13), C(18), C(20), C(29), C(30)
20		150.9 (*s*)	
21	1.29 – 1.30 (*m*)	31.3 (*t*)	C(19), C(22)
22	1.17 – 1.19 (*m*)	40.0 (*t*)	C(21)
23	0.86 (*s*)	27.8 (*q*)	C(3), C(4)
24	0.85 (*s*)	16.4 (*q*)	C(4), C(5), C(23)
25	0.84 (*s*)	15.7 (*q*)	C(1), C(9), C(10)
26	1.06 (*s*)	10.1 (*q*)	C(7), C(9), C(14)
27	1.41 (*s*)	14.9 (*q*)	C(8), C(14), C(15)
28	0.79 (*s*)	17.8 (*q*)	C(16), C(17), C(18), C(22)
29	4.57 (*s*), 4.68 (*s*)	109.3 (*t*)	C(18), C(30)
30	1.68 (*s*)	19.3 (*q*)	C(19), C(20), C(29)
1′		173.6 (*s*)	
2′	2.28 (*t*, J = 7.6)	34.8 (*t*)	C(1′), C(3′)
3′	1.68 – 1.70 (*m*)	25.0 (*t*)	
4′ – 15′	1.25 (br. *s*)	29.1 – 29.7 (*t*)	
16′	0.88 (*t*, J = 7)	14.11 (*q*)	

supported by the appearance of a signal due to a ester C=O group in the ^{13}C-NMR spectrum at 173.6 ppm. Furthermore, the signal at δ(H) 4.47 (*dd*, J = 11.2, 4.4, 1 H) was ascribable to H–C(3) in the triterpene moiety, close to the above mentioned C=O group as indicated by the ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra (*Fig. 2*). Although the ^{13}C-NMR spectrum displayed only 44 C-atoms instead of 46, the signals of the remaining 2 C-atoms must be overlapping with those of the flexible palmitate side chain. The unequivocal structure was achieved with fragments deduced from HSQC experiments. In summary, DEPT-135 signals of **1** were observed for eight Me, 23 CH_2, and eight CH C-atoms. The remaining C-atoms should be quaternary, since their signals were not observed in the DEPT-135 experiment. The ^{1}H,^{1}H-COSY, HSQC, and

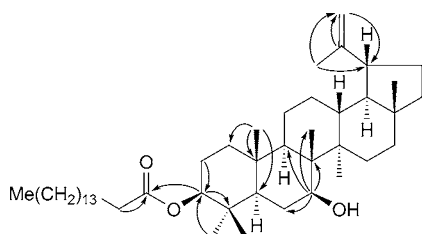

Fig. 2. *Key HMBCs* (H → C) *of compound* **1**

HMBC studies confirmed all the fragments and connectivities. Spectral data correlate well with literature data for a hydroxylated esterified lupine nucleus (lupeol) [9], and, thus, compound **1** was elucidated as (3*β*,7*β*)-7-hydroxylup-20(29)-en-3-yl hexadecanoate.

Other known compounds (*Fig. 1*), 7*β*,15*α*-dihydroxylup-20(29)-ene-3*β*-*O*-palmitate (**2**) [7], lupeol palmitate (**3**) [10], lupeol (**4**) [11], 3-oxolup-20(29)-ene (**5**) [12], ursolic acid (**6**) [13], cycloeucalenol (**7**) [14], stigmasterol (**8**) [15], *β*-sitosterol (**9**) [16], *β*-daucosterol (**10**) [17], quercetin (**11**) [18], quercetin 3-*O*-*α*-L-arabinoside (**12**) [19], quercetin 3-*O*-*α*-L-rhamnoside (**13**) [20], catechin (**14**) [21], gitoxigenin 3-*O*-*α*-L-rhamnoside (**15**) [22], gitoxigenin 3-*O*-*α*-D-glucoside (**16**) [23], digitoxigenin 3-*O*-*α*-L-rhamnoside (**17**) [24], were also identified by NMR and MS data, and chemical methods.

Compounds **1**–**7** and **11**–**17** were evaluated for their *in vitro* cytotoxicities against human leukemia cells (HL-60), human gastric cancer cells (SGC-7901), and human pancreatic cancer cells (PANC-1) using MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay [25]. The results are compiled in *Table 2*, revealing that compounds **4**, **6**, **11**, **15**–**17** exhibit effective cytotoxicities against these tumor cells.

Table 2. *Cytotoxic Activities of Compounds* **1**–**7** *and* **11**–**17**

Compound	IC_{50} [μg/ml]		
	PANC-1	HL-60	SGC-7901
1	> 80.00	> 80.00	> 80.00
2	> 80.00	> 80.00	> 80.00
3	> 80.00	> 80.00	> 80.00
4	43.21	63.24	41.05
5	> 80.00	> 80.00	> 80.00
6	12.80	16.58	13.12
7	63.42	18.45	21.32
11	41.19	17.36	46.73
12	> 80.00	> 80.00	> 80.00
13	> 80.00	> 80.00	> 80.00
14	> 80.00	> 80.00	> 80.00
15	21.21	27.83	17.56
16	34.20	15.32	23.11
17	24.52	9.50	12.59

In addition, the cytotoxicity of **4** was stronger than those of **5**, and **1** – **3** against HL-60, SGC-7901, and PANC-1 cells. Compound **4** has a free HO–C(3), while compound **5** bears a C(3)=O group, indicating that replacement the 3-OH group with an oxo group decreased activity. Compounds **4** and **1** – **3** differ in the substitution at C(3), compounds **1** – **3** were esterified at C(3), confirming that a free OH group at C(3) is essential for significant cytotoxicity of lupeol-based triterpenoids.

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Experimental Part

General. TLC: Silica gel *G* (*Qingdao Marine Chemical Factory*). Column chromatography (CC): silica gel (SiO_2, 100 – 200 or 200 – 300 mesh; *Qingdao Marine Chemical Factory*), *Sephadex LH-20* gel (*Amersham Pharmacia Biotech*), and *MCI* gel *CHP-20P* (75 – 150 μm; *Mitsubishi Chemical Co.*). M.p.: *WRS-1B* digital melting-point apparatus; uncorrected. Optical rotations: *JASCO-20* polarimeter. IR Spectra: *Nicolet 170SX* FT-IR spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR spectra: *Bruker* NMR spectrometer; at 400 or 600 (^1H), and 100 or 150 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Bruker APEXII* FT-MS spectrometer; in *m*/*z*. FAB-MS: *VG-ZAB-HS* mass spectrometer; in *m*/*z*. EI-MS: *HP-5988* mass spectrometer; in *m*/*z*.

Plant Material. The leaves of *Scurrula parasitica* L. parasitic on *Nerium indicum* Mill were collected in Taijiang District, Fuzhou, Fujian Province, P. R. China, in September 2012, and identified by Prof. *Y.-H. Z.*, Fujian Medical University. A voucher specimen (No. 2013022) was deposited with the Laboratory of the Natural Products, Fujian Medical University, P. R. China.

Extraction and Isolation. Dried and powdered leaves of *Scurrula parasitica* L. parasitic on *Nerium indicum* Mill (5 kg) were extracted three times with MeOH (3 × 10 l) at r.t. After evaporation of the solvent under reduced pressure, the residue was suspended in H_2O and extracted with petroleum ether (PE), AcOEt, and BuOH, successively. The residue of the PE layer (56.2 g) was fractionated by CC (SiO_2; PE/AcOEt 1 : 0 – 0 : 1) to yield ten fractions, *Frs. 1 – 10*, and compound **4** (67.4 mg) was isolated from *Fr. 1* (1.2 g). Compounds **1** (23.1 mg), **2** (62.4 mg), **3** (54.3 mg), **5** (108.7 mg) were isolated from *Fr. 2* (1.6 g). *Fr. 3* (2.3 g) was subjected to CC (SiO_2; PE/AcOEt 1 : 0 – 0 : 1) to furnish **7** (56.3 mg), **8** (45.4 mg), and **9** (35.2 mg). *Frs. 5* and *6* (2.1 g) was purified by repeated CC (SiO_2) to give compound **6** (32.1 mg).

The AcOEt layer (71.4 g) was fractionated by CC (SiO_2; PE/AcOEt 1 : 0 – 0 : 1) to yield eleven fractions, *Frs. 1 – 11*, and compounds **11** (103.5 mg) and **12** (112.3 mg) were isolated from *Fr. 6* (1.4 g) by repeated CC (SiO_2; PE/AcOEt 1 : 0 – 0 : 1) and CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1). *Fr. 9* (0.9 g) was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1) to afford **10** (97.2 mg). *Fr. 11* (0.8 g) was purified by repeated CC (SiO_2 and *Sephadex LH-20*; $CHCl_3$/MeOH 1 : 1) to give compounds **13** (32.1 mg) and **14** (46.4 mg).

The BuOH layer (136.4 g) was fractionated by CC (SiO_2; AcOEt/MeOH 1 : 0 – 0 : 1) to yield 17 fractions, *Frs. 1 – 17*. *Fr. 9* (5.6 g) was subjected to CC (*MCI*; H_2O/MeOH 1 : 0 – 0 : 1) to afford twelve fractions, *Fr. 1a* – *Fr. 12a*. *Fr. 9a* (0.7 mg) was purified by prep HPLC (column, *Kromasil* 250 × 10 mm, 5 μm; MeOH/H_2O 30 : 70) to yield **15** (36 mg), **16** (27 mg), and **17** (30 mg).

(3β,7β)-7-Hydroxylup-20(29)-en-3-yl Hexadecanoate (**1**). White amorphous powder. M.p. 103 – 105°. $[\alpha]^{25}_{D} = +41.0$ ($c = 0.50$, $CHCl_3$). IR: 3167, 2947, 2915, 1715, 1463, 1375, 717. ^1H- and ^{13}C-NMR: see *Table 1*. EI-MS: 681 ($[M + H]^+$), 665 ($[M - Me]^+$), 648, 426. HR-ESI-MS: 703.6010 ($[M + Na]^+$, $C_{46}H_{80}NaO_3^+$; calc. 703.6005).

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