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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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To cite this Article Ma, Zhong-Ze , Hano, Yoshio , Qiu, Feng , Shao, Gang , Chen, Ying-Jie and Nomura, Taro(2007) 'Triterpenoids from *Peganum nigellastrum*', Journal of Asian Natural Products Research, 9: 6, 575 — 578
To link to this Article: DOI: 10.1080/10286020701611636
URL: http://dx.doi.org/10.1080/10286020701611636

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Journal of Asian Natural Products Research, Vol. 9, No. 6, September 2007, 575–578

Triterpenoids from *Peganum nigellastrum*

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Two triterpenoids were isolated from the roots of *Peganum nigellastrum* Bunge. Their structures were elucidated as 3α-acetoxy-27-*trans*-caffeoyloxyolean-12-en-28-oic acid methyl ester (**1**) and 3-oxotirucalla-7, 24-dien-21-oic acid (**2**) on the basis of spectroscopic evidence. **1** is a new triterpene ester and **2** is a known compound isolated for the first time from genus *Peganum*.

Keywords: *Peganum nigellastrum*; Zygophyllaceae; Roots; Triterpenoid

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1. Introduction

The plant *Peganum nigellastrum* Bunge ('Luo-Tuo-Hao' in Chinese, Zygophyllaceae), commonly found in the northwest region of China, has been used in Chinese Traditional Medicine for the treatment of rheumatism, abscesses, and inflammatory diseases [1]. Pharmacological study of this plant revealed that the basic fraction showed potent anti-tumor activity [2]. We have previously reported several alkaloids from the aerial parts of the plant [3–5]. Among those alkaloids, luotonin A has unique pyrroloquinazolinoquinoline ring system, and showed cytotoxic activity against mouse leukemia P-388 cells and inhibitory activity against human topoisomerase II [6]. Recent study demonstrated that luotonin A stabilized the human DNA topoisomerase I-DNA covalent binary complex, affording the same pattern of cleavage as the structurally related topoisomerase I inhibitor camptothecin [7]. On the other hand, we isolated seven triterpenoids from the roots of *P. nigellastrum* and 3α, 27-dihydroxylup-20(29)-en-28-oic acid methyl ester exhibited significant topoisomerase II inhibitory activity [8]. In a continuation of the chemical studies on the same plant, two triterpenoids were isolated. The present paper describes the structural elucidation of both triterpenoids.

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020701611636

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2. Results and discussion

The ethanol extract of the dried roots was divided into hexane, benzene, chloroform, acetone and methanol fractions. The benzene fraction was purified by repeated silica gel column chromatography and ODS HPLC to give compounds **1** and **2** (figure 1). The known compound **2** was identified as 3-oxotirucalla-7, 24-dien-21-oic acid by comparison of its NMR, MS and physical data with those described in the literature [9], and **2** is isolated for the first time from genus *Peganum*.

Compound **1** was obtained as white amorphous powder, and gave dark green colouration with 5% $FeCl_3$ reagent on a TLC plate. The UV spectrum of **1** exhibited absorption maxima at 217, 244, 301 and 330 nm. The IR spectrum showed the presence of hydroxyl groups (3385 cm^{-1}), carboxyl groups (1712 and 1701 cm^{-1}), and aromatic group (1604 and 1516 cm^{-1}). The molecular formula, $C_{42}H_{58}O_8$, was determined by HR MALDI-TOF MS and confirmed by EI-MS (*m/z* 690, M^+), ^{13}C and DEPT NMR spectra (Table 1). The ^{13}C NMR spectrum of **1** revealed 42 carbon signals, which were ascribed to 7 × CH_3, 1 × OCH_3, 11 × CH_2, 10 × CH, ten quaternary carbons, and three carbonyl carbons (Table 1). In the ^1H NMR spectrum, AB type signals (δ 6.20 and 7.52, $J = 15.8$ Hz) due to the protons on a *trans*-disubstituted double bond and ABC type signals [δ 6.88 (d, $J = 8.2$ Hz), 6.96 (dd, $J = 1.8$ and 8.2 Hz), 7.07 (d, $J = 1.8$ Hz)] from three aromatic protons were noted. From the above UV, IR and ^1H NMR spectral data, the presence of a 3, 4-dihydroxycinnamoyl moiety in **1** was suggested. This was further confirmed by a very strong ion at *m/z* 163 and a series of ^{13}C signals (Table 1) similar to those of caffeic acid [10].

The ^1H NMR spectrum showed 6 tertiary methyl groups [δ 0.74, 0.83 (6H, s), 0.88, 0.92 and 0.96], a methyl proton signal of acetyl group (δ 1.94), a methoxyl group (δ 3.66), a —CH_2O- group (4.18 and 4.40, d, $J = 12.6$ Hz), a secondary alcohol group (δ 4.58, br s), and an olefinic proton signal (δ 5.63, t, $J = 3.0$ Hz). Assignments of ^1H and ^{13}C signals by 2D NMR revealed that **1** was an analogue of olean-12-en-28-oic acid. The locations of acetoxyl group at the C-3 position and caffeoyloxyl group at the C-27 position were substantiated on the basis of the HMBC spectrum. On the other hand, the equatorial 3β-H orientation at the C-3 position was clear from the *J* values (δ 4.58, br s, $W_{1/2} = 6$ Hz) and the ^{13}C chemical shift values of C-24 (δ 21.9) and C-5 (δ 50.2) [11]. These data suggested that the aglycone of **1** corresponds to an epimer of **3** [12], with only the C-3 stereochemistry being different. Thus, compound **1** was characterized as 3α-acetoxy-27-*trans*-caffeoyloxyolean-12-en-28-oic acid

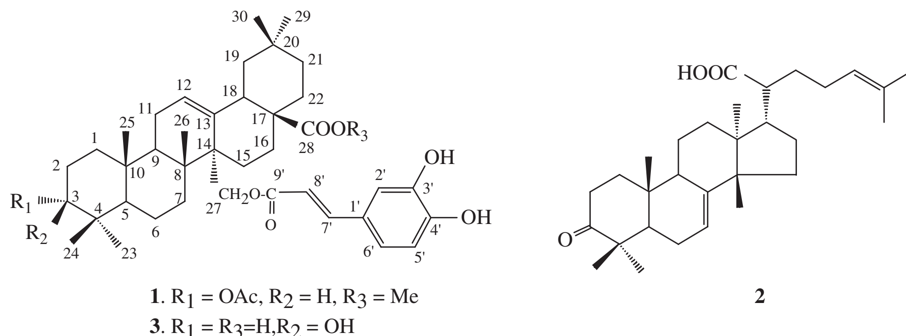

Figure 1. Structures of **1** and **2** from the roots of *P. nigellastrum*.

Table 1. ^{13}C NMR data of compound **1** ($CDCl_3$, 100 MHz).

C No	***1***	***3***^{a}	*DEPT*	*C No*	***1***	***3***^{a}	*DEPT*
1	33.8	38.6	CH_2	22	32.4	32.5	CH_2
2	22.6	26.8	CH_2	23	27.9	28.0	CH_3
3	78.5	78.8	CH	24	21.9	16.7	CH_3
4	36.5	38.8	C	25	15.5	15.6	CH_3
5	50.2	55.3	CH	26	18.1	18.0	CH_3
6	18.1	18.4	CH_2	27	66.2	65.9	CH_2
7	33.0	33.2	CH_2	28	178.4	180.8	C
8	40.2	40.0	C	29	33.0	33.0	CH_3
9	48.6	48.8	CH	30	23.6	23.6	CH_3
10	37.2	37.2	C	1′	127.6	126.9	C
11	23.9	24.0	CH_2	2′	114.2	114.0	CH
12	127.1	127.0	CH	3′	144.4	145.0	C
13	137.4	137.5	C	4′	146.4	147.6	C
14	45.3	45.4	C	5′	115.6	115.4	CH
15	23.6	23.6	CH_2	6′	122.3	122.0	CH
16	22.9	22.9	CH_2	7′	144.5	145.4	CH
17	46.6	46.2	C	8′	116.1	115.1	CH
18	41.3	41.1	CH	9′	167.3	167.8	C
19	44.7	44.9	CH_2	OCH_3	51.8	–	CH_3
20	30.6	30.6	C	$COCH_3$	171.3	–	C
21	33.8	33.8	CH_2	$COCH_3$	21.3	–	CH_3

a: **3** was recorded in $CDCl_3$-CD_3OD (10:1) [12].

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methyl ester. This proposed structure was supported by a comparison of the ^{13}C NMR data of **1** with those of **3** (Table 1).

3. Experimental section

3.1 General experimental procedure

Optical rotations were measured using a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Shimadzu 265 UV photometer. IR spectra were recorded on a JASCO FTIR 300 spectrophotometer. NMR spectra were acquired on a JEOL JNM EX-400 FTNMR spectrometer with TMS as internal standard. EI-MS spectra were obtained on a JEOL JMS-AM II 50 spectrometer. Diaion HP 20 (Mitsubishi Chemical Coporation, Tokyo, Japan) and Wakogel C-200 (silica gel, Wako Pure Chemical Co., Ltd., Osaka, Japan) were used for column chromatography. HPLC was carried on a SSC Flow System E-3100 (Senshu Scientific Co., Ltd, Tokyo, Japan) equipped with a SSC-3000B UV detector. Capcell Pak ODS column (10 X 250 mm) was used for HPLC at a flow rate of 2 ml/min.

3.2 Plant material

The roots of *P. nigellastrum* Bunge were collected in the suburb of Yenchuan city, Ningxia, China, in August 1997, and identified by Prof. Shirui Xing (Ningxia Institute for Drug Control). An authentic specimen (NX 970820-2) has been deposited in Shenyang Pharmaceutical University.

3.3 Extraction and Isolation

The air-dried roots of *P. nigellastrum* (4.8 kg) were finely cut and extracted three time with 95% ethanol (10 l) under reflux for 2 h in a water bath. The ethanol solution was evaporated *in vacuo* to give a residue (400 g) that was fractioned over Diaion HP-20 by successive elution with *n*-hexane, benzene, chloroform, acetone and methanol. The benzene fraction (80 g) was extracted twice with 60% ethanol (100 ml) at room temperature to yield an extract (15 g) that was chromatographed over silica gel (300 g), eluting with *n*-hexane with increasing amounts of ethyl acetate to afford fractions 1-160 (250 ml/fraction). The combined fractions 60-70, eluted with hexane-ethyl acetate (3:1) were successively purified with preparative TLC ($CHCl_3$-MeOH, 10:1) and ODS HPLC (MeOH-H_2O, 9:1) to give **1** (6 mg) and **2** (10 mg).

Compound **1** was obtained as white amorphous powder; 5% $FeCl_3$ reagent on a TLC plate: positive; $[\alpha]_D + 64$ (c = 0.10, $CHCl_3$); UV (MeOH) λmax (log ε): 217 (4.09), 244 (3.98), 301 (3.92), 330 (4.10) nm; IR (KBr) νmax: 3385, 2938, 2870, 1701, 1632, 1604, 1516, 1446, 1377, 1262, 1115, 982, 755 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.74 (3H, s), 0.83 (6H, s), 0.88 (3H, s), 0.92 (3H, s), 0.96 (3H, s), 1.94 (3H, s), 2.94 (1H, dd, $J = 5.0$ and 14.2 Hz), 3.66 (3H, s), 4.18 (1H, d, $J = 12.6$ Hz), 4.40 (1H, d, $J = 12.6$ Hz), 4.58 (1H, brs), 4.88 (1H, d, $J = 12.7$ Hz), 5.63 (1H, t, $J = 3.0$ Hz), 6.20 (1H, d, $J = 15.8$ Hz), 6.88 (1H, d, $J = 8.2$ Hz), 6.96 (1H, dd, $J = 1.8$ and 8.2 Hz), 7.07 (1H, d, $J = 1.8$ Hz), 7.52 (1H, d, $J = 15.8$ Hz); ^{13}C NMR data, see Table 1; EI-MS *m/z*: 690 $[M]^+$(0.3), 630 (1.6), 510 (21.4), 497 (13.8), 377 (30.5), 313 (10.1), 299 (18.6), 261 (16.0), 255 (28.7), 241 (32.4), 227 (24.5), 215 (24.2), 201 (100), 163 (64.2); Negative HR MALDI-TOF MS: 689.4052 $[M-1]^+$ (calcd for $C_{42}H_{57}O_8$: 689.4053).

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