Three New Triterpenoids from *Peganum nigellastrum*

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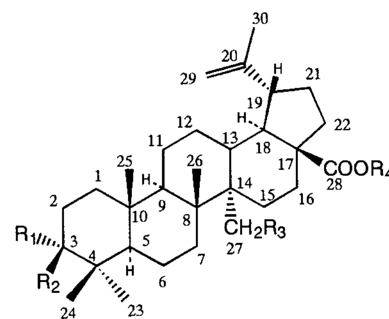
Three new triterpenoids, 3α,27-dihydroxylup-20(29)-en-28-oic acid methyl ester, 3α-acetoxy-27-hydroxylup-20(29)-en-28-oic acid methyl ester, and 3α-acetoxyolean-12-ene-27,28-dioic acid 28-methyl ester, were isolated from the roots of *Peganum nigellastrum* along with four known lupene-type triterpenoids. The structures of the new triterpenoids were determined by NMR spectroscopic means. The new triterpene, 3α,27-dihydroxylup-20(29)-en-28-oic acid methyl ester is a DNA topoisomerase II inhibitor (IC_{50} = 8.9 μM/mL).

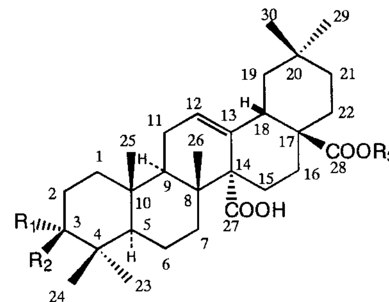
Peganum nigellastrum Bunge (Zygophyllaceae, Chinese name "Luo-Tuo-Hao") is distributed throughout Asia and is commonly found in the northwest region of China. The plant has been used as a Chinese traditional medicine for rheumatism, abscesses, inflammations, and a host of other ailments.[1] In 1988, Xiao et al. reported that the basic fraction of *P. nigellastrum* exhibited potent antitumor activity.[2] We have previously reported several alkaloids from the aerial parts of the plant.[3,4] It has also been reported that the main alkaloids of the aerial parts were different from those of the underground parts.[5,6] This led us to investigate the chemical constituents of the underground parts of the plant. This paper describes the structures of lupane and oleanane triterpenoids that have not been previously isolated from the aerial parts of the plant.

The ethanolic extract of the dried roots of *P. nigellastrum* was divided into *n*-hexane-, benzene-, chloroform-, acetone-, and methanol-soluble fractions. The *n*-hexane fraction was purified by repeated Si gel column chromatography to give compounds **1**−**3** and **5**−**7** (Figure 1). Analogous purification procedures of the benzene fraction gave compound **4** (Figure 1). The known compounds **4**, **5**, **6**, and **7** were identified as betulinic acid,[7] 3-*O*-acetylbetulinic acid,[7] 3-epibetulinic acid,[8] 3-*O*-acetylepibetulinic acid,[8] respectively, by comparison of their NMR, MS and physical data with those described in the literature.

Compound **1** was obtained as colorless needles, mp 132−134 °C. The molecular formula, $C_{31}H_{50}O_4$, was determined by HREIMS. The ^1H NMR spectrum of **1** showed five tertiary methyl groups (one of them shifted downfield to δ 1.67), a methoxyl group (δ 3.67), a secondary alcohol group (δ 3.38, t, J = 2.8 Hz), a hydroxymethyl group at δ 4.28 and 3.77 (each 1H, d, J = 12.5 Hz), and *exo*-methylene protons at δ 4.72 (1H, d, J = 1.6 Hz), 4.59 (1H, br s) (Table 1). The ^{13}C NMR spectrum of **1** revealed 31 carbon signals, which consist of 5 × CH_3, 1 × OCH_3, 12 × CH_2, 6 × CH, and seven quaternary carbons by DEPT analysis. Assignments of the ^1H and ^{13}C signals were performed by extended 2D NMR methods involving $^1H-^1H$ COSY, $^1H-^{13}C$ COSY, and HMBC spectra, which indicated **1** to be an analogue of 3-epibetulinic acid (Table 1). In the HMBC spectrum, the primary alcohol group resonating at δ 4.28 and 3.77 was correlated with C-8, C-13, and C-14,



1 $R_1=R_3=OH$, $R_2=H$, $R_4=CH_3$
2 $R_1=OAc$, $R_2=H$, $R_3=OH$, $R_4=CH_3$
4 $R_1=R_3=R_4=H$, $R_2=OH$
5 $R_1=R_3=R_4=H$, $R_2=OAc$
6 $R_1=OH$, $R_2=R_3=R_4=H$
7 $R_1=OAc$, $R_2=R_3=R_4=H$



3 $R_1=OAc$, $R_2=H$, $R_3=CH_3$
8 $R_1=R_3=H$, $R_2=OH$

Figure 1. Triterpenes (**1**−**7**) from the roots of *P. nigellastrum*.

indicating that the primary alcohol group was located at the C-27 position. Therefore, the structure of **1** was represented by 3α,27-dihydroxylup-20(29)-en-28-oic acid methyl ester. The NOESY spectrum of **1** also supported that **1** has the same stereochemistry as 3-epibetulinic acid (Figure 2).

Compound **2** was obtained as colorless needles, mp 224−226 °C. The molecular formula, $C_{33}H_{52}O_5$, was determined by HREIMS and confirmed by ^{13}C NMR and DEPT analyses. The ^1H and ^{13}C NMR data were closely related to those of **1**, except for additional signals arising from the acetoxyl group, which suggested **2** to be a monoacetate of **1**. This was also proved by an alkaline hydrolysis of **2**

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of **1**, **2**, and **3** in $CDCl_3$

carbon no.	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	33.8	1.24 m, 1.40 m	34.1	1.14 m, 1.42 m	33.8	1.25 m, 1.38 m
2	25.7	1.53 m, 1.96 m	22.9	1.59 m, 1.83 m	22.6	1.57 m, 1.83 m
3	76.5	3.38 (t, 2.8)	78.3	4.62 (br s)	78.1	4.60 (br s)
4	37.9		36.8		36.3	
5	49.6	1.26 m	50.4	1.28 m	50.0	1.18 m
6	18.5	1.38 m	18.0	1.26 m, 1.45 m	18.1	1.38 m
7	36.1	1.46 m, 1.57 m	35.5	1.43 m, 1.65 m	36.4	1.26 m, 1.65 m
8	42.0		41.7		40.2	
9	52.2	1.43 m	51.8	1.41 m	47.1	2.10 m
10	37.9		37.4		37.0	
11	21.3	1.46 m	21.0	1.45 m	22.7	1.93 m, 2.10 m
12	25.4	0.77 m, 1.66 m	25.0	0.78 m, 1.70 m	127.5	5.82 (dd, 2.8, 4.6)
13	39.3	2.31 (dt, 3.5, 13.7)	38.9	2.32 (dt, 3.6, 12.8)	136.8	
14	46.6		46.3		56.0	
15	23.5	1.16 m, 1.77 m	23.2	1.17 m, 1.79 m	24.2	1.52 m, 2.04 m
16	33.4	1.31 m, 2.30 m	33.1	1.38 m, 2.28 m	23.8	1.69 m, 1.94 m
17	56.7		56.3		47.5	
18	50.1	1.72 (t, 10.6)	49.8	1.75 (t, 10.3)	43.2	2.96 (dd, 3.3, 10.3)
19	47.2	3.00 (dt, 4.6, 10.6)	46.8	3.00 (dt, 4.0, 10.3)	43.6	1.13 m, 1.35 m
20	150.8		150.2		30.7	
21	30.8	1.36 m, 1.90 m	30.5	1.38 m, 1.90 m	33.5	1.16 m
22	37.1	1.42 m, 1.93 m	36.7	1.42 m, 1.92 m	31.8	1.47 m, 1.66 m
23	28.5	0.82 (3H, s)	27.7	0.87 (3H, s)	27.8	0.81 (3H, s)
24	22.4	0.92 (3H, s)	21.7	0.82 (3H, s)	21.7	0.86 (3H, s)
25	16.8	0.85 (3H, s)	16.4	0.87 (3H, s)	16.6	0.95 (3H, s)
26	16.6	0.94 (3H, s)	16.2	0.95 (3H, s)	18.2	0.85 (3H, s)
27	61.4	3.77, 4.28 (each d, 12.5)	61.0	3.82, 4.28 (each d, 12.4)	180.2	
28	177.0		176.6		178.0	
29	110.0	4.59 (br s), 4.72 (d, 1.6)	109.7	4.60 (br s), 4.73 (br s)	33.0	0.83 (3H, s)
30	19.8	1.67 (3H, s)	19.5	1.68 (3H, s)	23.6	0.90 (3H, s)
OCH_3	51.6	3.67 (3H, s)	51.3	3.68 (3H, s)	51.7	3.64 (3H, s)
CH_3CO			170.8		170.7	
CH_3CO			21.4	2.08 (3H, s)	21.2	2.04 (3H, s)

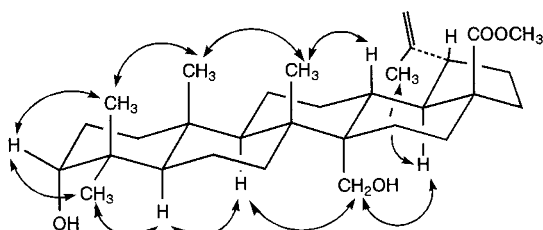

Figure 2. Significant NOE interactions of **1**.

yielding **1** and by a downfield shift of H-3 (δ 4.62) in **2** (Table 1). The detailed assignment of the ^{1}H and ^{13}C signals were performed by $^{13}C-^{1}H$ COSY and HMBC experiments. Accordingly the structure of **2** was established as 3α-acetoxy-27-hydroxylup-20(29)-en-28-oic acid methyl ester.

Compound **3** was obtained as colorless needles, mp 212–214 °C. The molecular formula, $C_{33}H_{50}O_6$, was determined by HREIMS and confirmed by ^{13}C NMR and DEPT analyses. The ^{13}C NMR spectrum of **3** revealed 33 carbon signals, which were ascribed to 7 × CH_3, 1 × OCH_3, 10 × CH_2, 5 × CH, seven quaternary carbons, and three carbonyl carbons (Table 1). The ^{1}H NMR spectrum of **3** showed an olefinic proton signal (δ 5.82, dd, J = 2.8, 4.6 Hz), a secondary alcohol group (δ 4.60, br s), a methoxyl group (δ 3.64), a methyl proton signal of an acetyl group (δ 2.04), and six tertiary methyl groups [δ 0.81, 0.83, 0.85, 0.86, 0.90, 0.95 (each 3H, s)] (Table 1). Assignments of the ^{1}H and ^{13}C signals by 2D NMR spectra revealed that **3** was an analogue of olean-12-en-28-oic acid. The location of an acetoxyl group at the C-3 position was substantiated by the HMBC spectrum, which showed a significant correlation between the H-3 and the acetyl carbonyl carbon. The remaining two carbonyl carbons, resonating at δ 178.0 and 180.2, were assigned to a carbomethoxyl group and a carboxyl group at the C-28 and C-27 positions, respectively, on the basis of the HMBC spectrum, in which the H-18 (δ 2.96) exhibits a correlation with the carbomethoxyl group (δ 178.0). On the other hand, the equatorial 3β-H orientation at the C-3 position was clear from the J values (δ 4.60, br s, $W_{1/2}$ = 6 Hz) and the ^{13}C chemical shift values of C-24 (δ 21.7) and C-5 (δ 50.0).[9] These data suggested that **3** corresponds to a diastereoisomer of cincholic acid (**8**),[10] with only the C-3 stereochemistry being different. Thus, compound **3** was characterized as 3α-acetoxyolean-12-ene-27,28-dioic acid 28-methyl ester. This proposed structure was supported by a comparison of the ^{13}C NMR data of **3** with those of cincholic acid (**8**).[10]

Recently, it has been reported that some lupane-type triterpenes show potent anti-HIV activity[11] and cytotoxic activity through inhibition of DNA topoisomerase II.[12] Compounds **1**–**3** were tested for inhibitory activity against human topoisomerase II. Only compound **1** showed significant activity (IC_{50} 8.9 μM, as compared to ICRF-193,[13] IC_{50} 13.9 μM).

The present study is the first report of pentacyclic triterpenoids in the genus *Peganum*, and these compounds were not detected in the aerial parts of *P. nigellastrum*. Furthermore, it should be noted that both 3α- and 3β-stereochemistries at the C-3 position of the triterpenes coexist in *P. nigellastrum*.

Experimental Section

General Experimental Procedures. Melting points were determined by a Yanaco micro-melting point apparatus MP-500V and are uncorrected. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO FTIR 300 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a JEOL JNM EX-400 FTNMR spectrometer (400 MHz) with tetramethyl-

silane (TMS) as internal standard. EIMS spectra were recorded on a JEOL JMS-AM II 50 spectrometer. Wakogel C-200 and B-5 FM (Si gel, Wako Pure Chemical Co., Ltd., Osaka, Japan) were used for column chromatography and TLC, respectively.

Plant Material. The underground parts of *P. nigellastrum* Bunge were collected in the suburb of Yenchuan City, Ningxia, China, in August 1997, and identified by Prof. Shi-Rui Xing. An authentic specimen (NX 970820-2) has been deposited in Shenyang Pharmaceutical University.

Extraction and Isolation. The air-dried roots of *P. nigellastrum* (4.8 kg) were finely cut and then extracted three times with 95% EtOH (10 l) under reflux for 2 h in a water bath (bath temperature 100 °C). The EtOH solution was evaporated in vacuo to give a residue (400 g) that was fractionated over Diaion HP-20 by successive elution with *n*-hexane, C_6H_6, $CHCl_3$, Me_2CO, and MeOH. The *n*-hexane-soluble fraction (39 g) was extracted twice with 70% EtOH (100 mL) at room temperature for 6 h, yielding a 7-g fraction, which was chromatographed over Si gel (300 g) using *n*-hexane with increasing amounts of EtOAc to prepare fractions 1−120 (200 mL/fraction). The combined fractions 16−18, eluted with *n*-hexane−EtOAc (20:1), were crystallized from $CHCl_3$ to give **5** (25 mg). The combined fractions 19−21, also eluted with *n*-hexane−EtOAc (20:1), were treated in the same way to afford **7** (120 mg). The combined fractions 30−36 and 42−45, eluted with *n*-hexane−EtOAc (10:1), were each crystallized from $CHCl_3$ to give **6** (14 mg) and **2** (250 mg), respectively. Using the same procedure, the combined fractions 68−72, eluted with *n*-hexane−EtOAc (5:1), gave **3** (15 mg), and combined fractions 96−100, eluted with *n*-hexane−EtOAc (2:1), gave **1** (16 mg).

The C_6H_6 fraction (80 g) was extracted twice with 60% EtOH (100 mL) at room temperature for 6 h to yield an extract (15 g) that was chromatographed over Si gel (300 g), eluting with *n*-hexane with increasing amounts of EtOAc to afford fractions 1−160 (250 mL/fraction). The combined fractions 20−23, eluted with *n*-hexane−EtOAc (5:1), were crystallized from $CHCl_3$ to give **4** (5 mg). Compounds **4**−**7** were identified as betulinic acid,[7] 3-*O*-acetylbetulinic acid,[7] 3-epibetulinic acid,[8] 3-*O*-acetylepibetulinic acid,[8] respectively, by comparison of NMR, MS, and physical data with those described in the literature.

3α,27-Dihydroxylup-20(29)-en-28-oic acid methyl ester (1): colorless needles ($CHCl_3$), mp 132−134 °C; $[\alpha]_D$ −20° (*c* 0.11, $CHCl_3$); IR (KBr) ν_{max} 3433, 2946, 2871, 1729, 1643, 1452, 1387, 1164, 989, 885 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 486 $[M]^+$ (2.0), 468 $[M - H_2O]^+$ (17.3), 455 $[M - CH_2OH]^+$ (68.8), 437 $[M - CH_2OH - H_2O]^+$ (21.1), 207 (75.6), 189 (100); HREIMS *m*/*z* 486.3702 (calcd for $C_{31}H_{50}O_4$, 486.3709).

3α-acetoxy-27-hydroxylup-20(29)-en-28-oic acid methyl ester (2): colorless needles ($CHCl_3$), mp 224−226 °C; $[\alpha]_D$ −42° (*c* 0.20, $CHCl_3$); IR (KBr) ν_{max} 3558, 2942, 1720, 1642, 1451, 1376, 1255 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 528 $[M]^+$ (0.4), 510 $[M - H_2O]^+$ (5.9), 497 $[M - CH_2OH]^+$ (49.2), 437 (34.9), 201 (66.4), 189 (100); HREIMS *m*/*z* 528.3824 (calcd for $C_{33}H_{52}O_5$, 528.3815).

Alkaline Hydrolysis of 2. A mixture of **2** (43 mg) in 10% aqueous NaOH (3 mL) and MeOH (7 mL) was refluxed for 2 h. The solvent was evaporated in vacuo, and the residue was extracted with $CHCl_3$ (10 mL × 3). The combined organic solvent was evaporated and purified by preparative TLC [$CHCl_3$−EtOAc (5:1)] to give **1** (34 mg).

3α-Acetoxyolean-12-ene-27,28-dioic acid 28-methyl ester (3): colorless needles (acetone), mp 212−215 °C; $[\alpha]_D$ +21° (*c* 0.15, $CHCl_3$); IR (KBr) ν_{max} 3500−2500 (COOH), 2947, 2866, 1730, 1464, 1386, 1373, 1244 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 542 $[M]^+$ (0.1), 524 $[M - H_2O]^+$ (0.4), 498 (1.8), 482 (21.8), 467 (10.3), 292 (9.4), 275 (18.4), 249 (10.6), 233 (51.4), 190 (100); HREIMS *m*/*z* 542.3605 (calcd for $C_{33}H_{50}O_6$, 542.3607).

Topoisomerase II Assay Procedure. The unknotting assays with human topoisomerases II (TopoGEN, Columbus, OH) were conducted as described previously.[14]

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