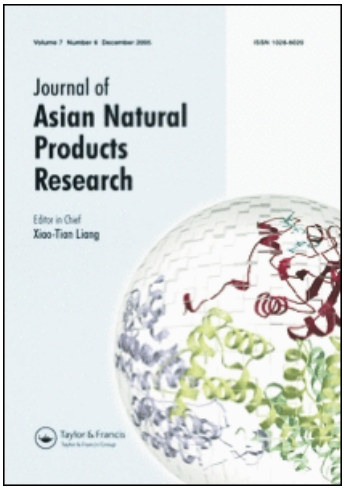

Journal of Asian Natural Products Research

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 Xu, YING-QIAN , Yao, ZHI , Hu, JUN-YI , Teng, JIE , Takaishi, YOSHIHISA and Duan, HONG-QUAN(2007) 'Immunosuppressive terpenes from *Prinsepia utilis*', Journal of Asian Natural Products Research, 9: 7, 637 — 642
To link to this Article: DOI: 10.1080/10286020600979589
URL: http://dx.doi.org/10.1080/10286020600979589

Journal of Asian Natural Products Research, Vol. 9, No. 7, October–November 2007, 637–642

Immunosuppressive terpenes from *Prinsepia utilis*

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(Received 16 March 2006; revised 14 June 2006; in final form 24 June 2006)

Two new hemiterpenes, utililactone (**1**) and epiutililactone (**2**), along with nine known compounds (**3**–**11**), were isolated from the leaves of *Prinsepia utilis*. Their structures were elucidated on the basis of spectroscopic data. The isolated compounds showed significant immunosuppressive activities.

Keywords: *Prinsepia utilis*; Utililactone; Epiutililactone; Immunosuppressive activity

1. Introduction

Prinsepia utilis Royle is a shrub plant growing at an altitude of 1000–2500 m in the south of China and India. It has been used in folk medicine to treat various skin diseases and rheumatism [1]. Pharmacological research indicates that the extract of *P. utilis* has antioxidative activity [2], and the hydrocyanic acid [3], fatty oil [4] as well as prinsepiol [5] have been isolated from this plant. In our search for bioactive compounds from medicinal herbs, we have studied the chemical constituents and reported a hemiterpene from *P. utilis* [6]. This paper deals with the isolation and structure elucidation of two new hemiterpenes, named utililactone (**1**) and epiutililactone (**2**), together with nine known compounds (**3**–**11**) from *P. utilis*. An immunosuppressive bioassay was carried out, and compounds **3**–**7** show significant inhibitory effect on lymphocyte transformation.

2. Results and discussion

The ethyl acetate-soluble fraction from the leaves of *Prinsepia utilis* was separated by repeated silica gel column chromatography, Toyopearl HW-40 and preparative HPLC to give

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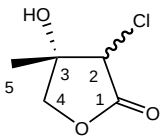
Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020600979589

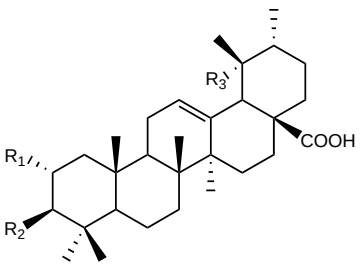
two new hemiterpenes, utililactone (**1**) and epiutililactone (**2**), as well as nine known compounds (**3**–**11**).

Utililactone (**1**) was obtained as colourless prisms, exhibiting a molecular ion peak at *m/z* 150.0065 $[M]^+$ (HREI-MS), indicating a molecular formula of $C_5H_7ClO_3$ for **1**. The EI-MS spectrum of **1** revealed the intensity of the $[M]^+$ peak (3.70%), $[M + 2]^+$ isotope peak (1.22%) and a fragment ion peak at *m/z* 115 $[M - 35]^+$ indicating the presence of a chlorine atom in **1**. The IR spectrum showed the presence of hydroxyl group (3498 cm^{-1}) and γ-lactone ring (1791 cm^{-1}). The ^1H NMR, ^{13}C NMR and HSQC spectral data of **1** revealed the presence of one tertiary methyl group [δ_C 20.4,δ_H 1.47 (3H, *s*)], one oxygenated methylene [δ_C 77.8, δ_H 4.17 (1H, *d*, $J = 9.5$ Hz), 4.23 (1H, *d*, $J = 9.5$ Hz)], one methine [δ_C 60.5,δ_H 4.21 (1H, *s*)], in addition to one oxygenated quaternary carbon (δ_C 77.2) and one carbonyl carbon (δ_C 173.8). In the HMBC spectrum, the methyl proton signal at δ 1.47 (H-5) correlated with the carbon signals at δ 77.8 (C-4), 77.2 (C-3), and 60.5 (C-2), and the signal at δ 4.21 (H-2) correlated with the signals at δ 173.8 (C-1) and 77.2 (C-3). From the above evidence, compound **1** was deduced to be 2-chloro-3-hydroxy-3-methyl-γ-butyrolactone (figure 1), which was an analogue of the natural product of (−)(*S*)-3-methyl-γ-butyrolactone [7].

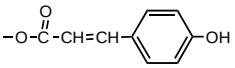


1 2*R*, 3*S*

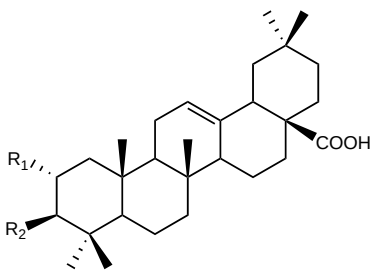
2 2*S*, 3*S*



	R_1	R_2	R_3
3	H	OH	H
5	OH	OH	H
7	H	OH	OH
8	OH	OH	OH

P-coumaroyl: 

	R_1	R_2	R_3
10	OH	*trans-p*-coumaroyl	OH
11	OH	*cis-p*-coumaroyl	OH



4 R_1 = H, R_2 = OH

6 R_1 = R_2 = OH

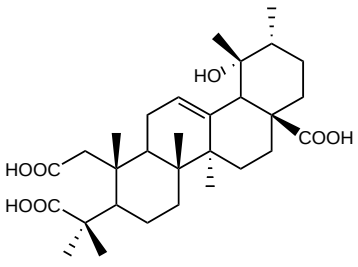


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Figure 1. The structures of **1**–**11**.

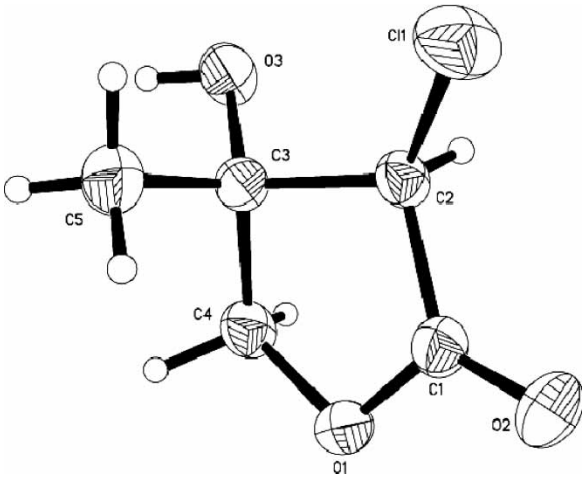

Figure 2. The ORTEP view of **1**.

In order to confirm the structure of **1**, an X-ray crystallographic analysis was undertaken. Compound **1** was determined as (+)-(2*R*, 3*S*)-2-chloro-3-hydroxy-3-methyl-γ-butyrolactone, and its ORTEP drawing was shown in figure 2.

Epiutililactone (**2**) had a molecular ion peak at *m/z* 150.0056 $[M]^+$ (HREI-MS), indicating a molecular formula of $C_5H_7ClO_3$ for **2**. The carbon framework of **2** was readily assignable to be the same as that of **1** by the ^{1}H NMR and ^{13}C NMR spectral data comparison (table 1). From the above evidence, compound **2** was deduced to be a diastereoisomer of **1**. The chemical shift of methyl group (C-5) was similar to that of **1** [δ_H 1.47 (s), **1**; δ_H 1.49 (s), **2**], however, the chemical shift of methine proton shifted to down field [δ_H 4.21 (s), **1**; δ_H 4.42 (s), **2**]. Therefore, compound **2** was deduced to be (+)-(2*S*, 3*S*)-2-chloro-3-hydroxy-3-methyl-γ-butyrolactone.

Nine known compounds were identified by their spectroscopic data in comparison with literature values as follow: ursolic acid (**3**) [8], oleanolic acid (**4**) [8], corosolic acid (**5**) [9], maslinic acid (**6**)[10], pomolic acid (**7**) [11], tormentic acid (**8**) [12], cecropiacic acid (**9**) [13], 3-*O-trans-p*-coumaroyltormentic acid (**10**) [12], 3-*O-cis-p*-coumaroyltormentic acid (**11**) [12].

In the search for immunosuppressive substances, we examined the immuno-inhibitory effect of isolated compounds on lymphocyte transformation [14,15]. Compounds **3**–**7** showed significant inhibitory effect ($P < 0.05$, $n = 6$) on lymphocyte transformation by comparing with a reference compound dexamethasone (table 2).

Table 1. ^{1}H NMR and ^{13}C NMR spectral data of **1** and **2**.

	1 *($CDCl_3$)*		***2*** *($CDCl_3$)*	
No.	^{13}C	$^{1}H^{\dagger}$	^{13}C	$^{1}H^{\dagger}$
1	173.8	–	170.5	–
2	60.5	4.21 (1H, s)	62.0	4.42 (1H, s)
3	77.2	–	75.0	–
4	77.8	4.17, 4.23 (each 1H, d, 9.5)	75.8	4.18, 4.42 (each 1H, d, 9.8)
5	20.4	1.47 (3H, s)	22.2	1.49 (3H, s)

† The chemical shifts of proton signals were read by HSQC spectrum.

Table 2. Inhibitory effects of compounds **1**–**11**.

	Inhibition (%)		
Compound	*80 μg/ml*	*20 μg/ml*	*5 μg/ml*
1	10.50	9.45	6.05
2	24.49	18.62	17.09
3	52.74	51.03	3.42
4	51.20	48.50	7.50
5	49.77	38.93	0.67
6	62.56	55.98	4.84
7	53.20	44.50	14.50
8	20.83	8.31	4.72
9	17.47	16.84	12.63
10	26.26	4.58	5.22
11	45.55	37.25	0.22

Inhibition ratio of dexamethasone = 41.62% (50 μg/ml).

3. Experimental

3.1 General experimental procedures

NMR analysis of samples were performed with a Bruker AVANCE 300 instrument (^{1}H 300 MHz, ^{13}C 75 MHz), both with teramethylsilane as an internal standard. HREI-MS data and EI-MS data were obtained on a JEOL JMS-SX102A and VG ZAB-HS (70 eV) instrument, respectively. Column chromatography was performed on silica-gel (Qingdao Haiyang Chemical Co. Ltd) and Toyopearl HW-40 (Tosoh). HPLC was a Jasco Gulliver Series with PU-1580 (pump), RI-1530 and UV-1575 (detector). Preparative HPLC column was used as follows: ODS (YMC-Pack ODS-A, SH-343-5), GPC (Shodex, Asahipak GS-310, 20G, MeOH). IR spectra were recorded on a FTS3000 Infrared Fourier Transform spectrometer (Bio-Rad). Optical rotations were measured with a MC 241 digital polarimeter (Perkin–Elmer).

3.2 Plant material

Prinsepia utilis Royle was collected from Kunming, Yunnan province of China in August 2002 and identified by Dr Xi-kui Liu, Kunming Institute of Botany, Chinese Academic of Sciences. A voucher specimen (D20020804) is deposited in the School of Pharmacy, Tianjin Medical University, China.

3.3 Extraction and isolation

The dried leaves (1.3 kg) of *Prinsepia utilis* Royle were crushed and extracted three times with EtOH (95%, 10 L each) at 60°C for 6 h. The EtOH extract was concentrated under reduced pressure to give a residue (190 g), which was suspended in H_2O, and then successively partitioned with petroleum ether, EtOAc and n-BuOH. The EtOAc layer was concentrated to afford a residue (28 g), which was subjected to column chromatography with silica gel, and was eluted with solvents of increasing polarity [petroleum ether/EtOAc (3:1, 2:1, 1:1, 1:2, 1:3), EtOAc, EtOAc/MeOH (19:1, 10:1), MeOH] to yield 16 fractions (fr. 1–16). Fraction 7 (2.5 g) was chromatographed on Toyopearl HW-40 ($CHCl_3$/MeOH 2:1) to give five fractions (fr. 7.1–7.5). Fraction 7.2 (308 mg) was purified by HPLC (ODS, MeOH) to give **3** (6.9 mg),

4 (50 mg) and **7** (13 mg). Fraction 7.5 (320 mg) was purified by HPLC (ODS, MeOH/H_2O 8:2, and then 6:4) to give **1** (56.2 mg) and **2** (24.5 mg). Fraction 10 (1.9 g) was further chromatographed on a silica gel column [$CHCl_3$/MeOH (95:5, 9:1, 85:15)] to give six fractions (fr. 10.1–10.6). Fraction 10.2 (456 mg) was chromatographed on HPLC (GPC, MeOH) to give nine fractions (fr. 10.2.1–10.2.9). Fraction 10.2.5 (68 mg) was separated by HPLC (ODS, MeOH/H_2O 9:1) to give **5** (40 mg), **6** (4.8 mg) and **8** (10.5 mg). Fraction 10.2.8 (96 mg) was separated by HPLC (ODS, MeOH-H_2O 9:1) to give **10** (15.5 mg) and **11** (4.8 mg). Fraction 10.2.9 (110 mg) was separated by HPLC (ODS, MeOH/H_2O 8:2) to give **9** (13.2 mg).

3.3.1 Utililactone (1). Obtained as colourless prisms. mp 89–91°C. $[\alpha]_D^{25}$ + 26.9 (*c* 1.7, MeOH). IR (KBr) λ_{max} cm^{-1}: 3499, 2982, 2952, 1791, 1479, 1441, 1397, 1353, 1264, 1218, 1148, 1117, 1004, 917, 862. EI-MS: *m*/*z* 152 $[M + 2]^+$(1.22), 150 $[M]^+$(3.7), 134 (0.9), 132 $[M - H_2O]^+$(2.7), 115 $[M - Cl]^+$(3.8), 94 (10.3), 92 (29.6), 85 (42.9), 78 (16.1), 76 (47.7), 57 (23.6), 43 (100), 39 (19.6). HREI-MS *m*/*z* 150.0065 $[M]^+$ (calcd for $C_5H_7ClO_3$, 150.0084). ^1H NMR and ^{13}C NMR ($CDCl_3$) data are listed in table 1.

3.3.2 Epiutililactone (2). Obtained as viscous syrup. $[\alpha]_D^{25}$ + 39.1 (*c* 1.7, MeOH). The main absorption bands of IR and EI-MS of **2** were the same as that of **1**. HREI-MS *m*/*z* 150.0056 $[M]^+$ (calcd for $C_5H_7ClO_3$, 150.0084). ^1H NMR and ^{13}C NMR ($CDCl_3$) data are listed in table 1.

3.4 X-Ray crystallographic analysis data of 1

A monoclinic crystal was obtained from a solvent system of $CHCl_3$. Crystal data: $C_5H_7ClO_3$, $M_r = 150.56$, monoclinic. Crystal size = $0.24 \times 0.20 \times 0.16$ mm. Cell parameters: $a = 6.513(3)$ Å, $b = 5.159(2)$ Å, $c = 9.839(5)$ Å, $V = 328.4(3)$ Å^3, space group $P2_1$ ($Z = 2$). Data collection was performed on a SMART (Bruker, 1997), the structure was resolved by direct methods (SHELXS-97), and the final R and R_w values were 0.0418 and 0.1085 for 1475 observed reflections.

Acknowledgements

The Project was sponsored by the Scientific Research Foundation of Tianjin Medical University.

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