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Coumarin dimer and sesquiterpene lactones from *Carpesium lipskyi* Winkl

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Carpesilipskyin **1**, a novel coumarin dimer which possesses a unique isopropyl side chain, together with two new sesquiterpene lactones, carabrol isovalerate **2** and 2′-hydroxy-4′,6′-dimethoxy-benzoylcarpesiolin **3** have been isolated from the methanol extract of the aerial parts of the title herbal plant followed by structure elucidation on the basis of spectral data.

Keywords: *Carpesium lipskyi*, carpesilipskyin, carabrol isovalerate, 5α,6α,10β-2′-hydroxy-4′,6′-dimethoxy-benzoylcarpesiolin

C. lipskyi Winkl (Asteraceae) growing in north-western part of China is highly valued for its medicinal properties in the local area. A decoction of the aerial parts of the plant is drunk as a substitute for tea to treat hepatitis type C and fungal infections since ancient times. However, upto now no systemic chemical investigation has been carried out although the aerial parts of the same species had been studied chemically to result in the isolation of carpelipine A and B[1]. As part of the continuing investigation of Asteraceae species[2,3], an earlier incomplete chemical investigation of the species prompted the present further study of this valuable folk remedy collected in Gansu Province, China, the results of which are described in this paper.

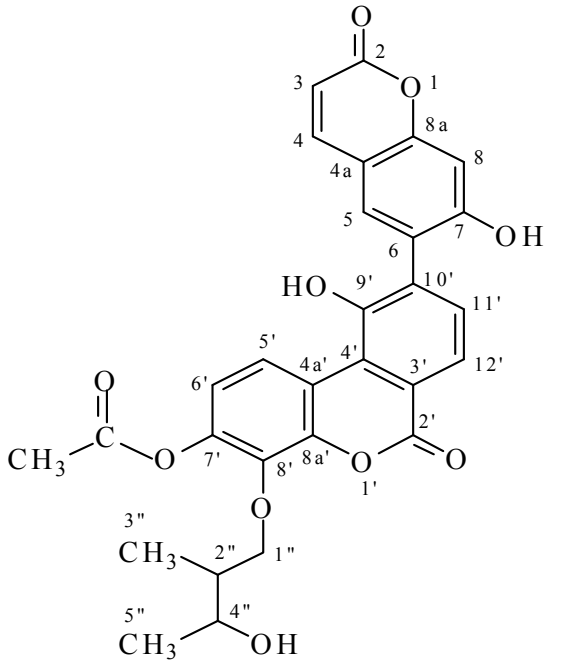
Results and Discussion

A methanol extract prepared from the aerial parts of *C. lipskyi* Winkl was fractionated and subjected to different types of column chromatography, as a result of which a new coumarin dimer **1** together with two new sesquiterpene lactones, carabrol isovalerate **2**, and 5α,6α,10β-2′-hydroxy-4′,6′-dimethoxy-benzoyl-carpesiolin **3** were obtained. In addition,6,7-dihydroxy coumarin and 6-methoxy-7-hydroxy-coumarin, 2-acetyl-3,5-dimethoxy phenol which were widely distributed in the genus *Artemisia* were also isolated. The spectral characteristics of known compounds including IR, ^{1}H and ^{13}C NMR data were identical with those previously described in the literature[4].

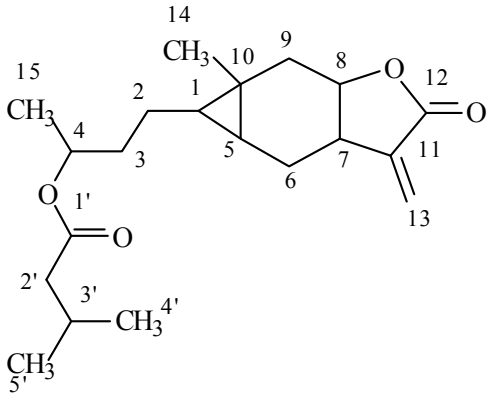
Compound **1** was isolated as pale yellow needle shaped crystals, the molecular formula of which was determined to be $C_{29}H_{24}O_{10}$ on the basis of positive HREIMS and ^{13}C NMR. The EIMS of **1** showed a major fragment at [M-$C_5H_8O_3$], besides two other fragments at [M-$C_5H_{11}O$] and [M-$CH_3C{=}O$], respectively. The compound showed from its ^{1}H, ^{13}C and DEPT NMR spectra the presence of three methyls, eight aromatic methines, three carbonyls, two aliphatic methines, one aliphatic methylene, and twelve quaternary carbons. The HMQC spectrum of **1** revealed the direct attachment between protons and carbons, and the HMBC spectrum gave long-range correlations between ^{1}H and ^{13}C through three or two bonds. All quaternary carbons were assigned from the analysis, and thus, the elucidation of the structural skeleton could be achieved.

The HMBC NMR spectrum showed key correlation contours between H-5 (δ 6.78) and C-10′ (δ 127.0), H-1″ (δ 4.45, and 4.30) and C-8′ (δ 143.8), H-6′ (δ 7.53) and AcCO (δ 168.9). These correlations, in particular, indicated that the C-6 of the ″upper″ coumarin unit was connected to C-10′ of the ″lower″ coumarin unit, and the side chain was linked between C-8′ and C-1″, and the acetyl group was located at 7′-OH simultaneously. All the other signals which appeared in the spectrum of **1** were assigned coherently.

The molecular formula of compound **2** was deduced to be $C_{20}H_{30}O_4$ by positive HRFABMS. The



1



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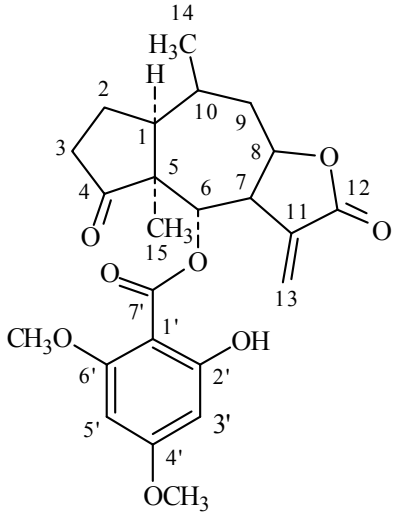

3

Figure 1 — Structures of three novel compounds

positive HRFABMS of **2** showed $[M+Na]^+$, and $[M+H]^+$ at *m/z* 357 and 335, respectively. The ^{1}H NMR spectrum of **2** was very similar to that of carabrol[5]. The only difference between **2** and carabrol lies that a set of isovalerate signal appeared on the ^{1}H NMR spectrum of **2**, besides the original carabrol proton signals. The above analysis indicated that compound **2** was carabrol isovalerate (**Figure 1**).

Compound **3** was isolated as colourless prisms. The molecular formula of **3** was determined as $C_{24}H_{28}O_8$ by positive HRFABMS. The positive HRFABMS of **3** showed $[M+Na]^+$, and $[M+H]^+$ at *m/z* 467 and 445, respectively. The proton NMR spectrum of **3** matched almost exactly with that of carpesiolin identified by Maruyama[5,6], except that an additional set of benzoyl signal substituted by a hydroxy and two methoxy groups, which appeared at δ 5.92 (5′-H), 6.05 (3′-H), 3.82 and 3.86 (each 3H, MeO-×2), respectively. The ^{13}C NMR spectrum was also in accord with the above analysis, and especially the HMBC spectrum of **3**

Table I — Major HMBC correlations observed in the spectra of compounds **1**, **2** and **3**

Compd	Positions	^{1}H	^{13}C	Correlated ^{13}C in HMBC
1	5	6.78	120.4	10', 4a, 8a, 7, 4
	1″	4.30 and 4.45	62.3	8', 2″, 3″, 4″
	6'	7.53	120.9	AcCO, 5', 4a', 7', 8'
2	1	0.41	28.5	2, 3, 5, 6, 9, 10
	13	5.45 and 6.11	148.8	11, 7,12
3	6	4.09	74.1	C=O, 5, 1, 7, 8, 11
	13	5.77 and 6.21	140.3	11, 12, 7, 6
	5'	6.07	105.7	4', 6', '1, 3'

which showed a significant correlating contour between a carbonyl group (δ 166.1) and δ 4.09 (C_6-H). This confirmed once again that the substituted benzoyl group was attached to C_6-OH, even if just this only possible position could be linked to each other between the two units of the compound. The *cis* ring junction at C-1 and C-5 was confirmed from the NOESY correlation between H-3/5-CH_3, H-3/H-7 and 5-CH_3 which indicated that the H-1 proton and the 5-CH_3 were α-oriented. The NOESY correlation between 6-H and 7-H, as well as 10-H and 1-H showed 6-O and 10-CH_3 were α- and β-oriented respectively. So the structure of **3** (**Figure 1**) was elucidated undoubtedly to be 5α,6α,10β-2′-hydroxy-4′,6′-dimethoxy-benzoylcarpesiolin. The major HMBC correlations of the three compounds are summarized in **Table I**.

Experimental Section

Melting points were determined using a Fisher-Johns apparatus and are uncorrected. IR spectra were obtained in KBr discs on a Perkin-Elmer 577 spectrometer. UV-Vis spectra were recorded on a Shimadzu 160 UV-Vis spectrometer. Optical rotations were measured on a JASCO DIP 360 digital polarimeter. All NMR experimental data were recorded on a Bruker DMX 500 or Varian Unity INOVA spectrometers using CD_3OD or $CDCl_3$ as solvent (500 or 300 MHz for ^{1}H and 125 or 75 MHz for ^{13}C NMR) using tetramethylsilane (TMS) as an internal standard. FABMS spectra were obtained on a VG 70-VSEQ mass spectrometer with a direct inlet system using PEG 600/glycerol as a matrix; EIMS spectra were determined on a JOEL JMS-SX 102A spectrometer at ionization energy of 70 eV. Silica gel (200-300 mesh) was used for column chromatography. Silica gel GF_{254} (400-450 mesh) for TLC was produced by Qingdao Marine Chemical Factory, Qingdao, China. Sephadex LH-20 was from Pharmacia Biotech, Sweden. All other chemicals used to carry out the experiments were of analytical grade.

Plant material. The aerial parts of *C. lipskyi* Winkl were collected in July 2002 from MaHan Mountain, Gansu Province, China, and identified by Prof. Z. Y. Zhu. A voucher specimen registered under the number YZ-0296201 was deposited in the Herbarium of Lanzhou University, Lanzhou 730000, PR China.

Extraction and isolation

Air-dried aerial parts of *C. lipskyi* Winkl were thoroughly crushed and the extraction initiated with MeOH-Et_2O-petroleum ether (1:1:1), followed by defatting with MeOH in the refrigerator. The cold methanol soluble part was subsequently concentrated in vacuum to a deep green black residue (43 g) which was chromatographed over 700 g silica gel using petroleum ether containing increasing amounts of Et_2O, and then followed with Et_2O-MeOH gradients to yield 100 crude fractions (F-1~F-100) (0.5 litre each), which were collected and monitored by TLC. F-1~F-3 contained nothing of interest. F-18~F-23 were collected together, and preparative TLC of one-tenth of it (petroleum ether-Et_2O, 17:1, double elution) gave 70 mg carabrol isovalerate. F-25~F-28 were subjected to MPLC on silica gel using $CHCl_3$ containing increasing amount of MeOH as eluant to give 2-acetyl-3,5-dimethoxy phenol as colourless needle shaped crystals (300 mg). Half of the amount of F-29~F-30 was subjected repeatedly to column chromatography over silica gel to give 6,7-dihydroxy coumarin (30 mg), 6-methoxy-7-hydroxy-coumarin (55 mg) as light yellow needles respectively. F-50~F-65 was again separated by column chromatography

over silica gel and colourless prisms and pale yellow crystals were obtained which was further purified over a Sephadex LH-20 column using MeOH as eluent to yield 2′-hydroxy-4′,6′-dimethoxy-benzoyl-carpesiolin (70 mg) and carpesilipskyin (55 mg) successively.

Compound **1**: pale yellow needles from MeOH; m.p. 125-26°C; $[\alpha]_D^{20}$+70° (*c* 0.1, MeOH); UV-Vis: nm (logε) 220 (4.95), 274 (4.77), 340 (4.47); IR (KBr): 3438 (OH), 1740 (C=O), 1400~1620 cm^{-1}(ϕ-C=C-); ^{1}H NMR (CD_3OD): δ 0.93 (3H, d, *J*=7.20 Hz, H-5″), 0.98 (3H, d, *J* =7.2 Hz, H-3″), 2.17 (3H, s, CH_3-C=O-), 3.32~4.09 (2H, m, H-2″, 4″), 4.27 (1H, brd, *J*=11.01 Hz, H-1″), 4.45 (1H, brd, *J*=10.91 Hz, H-1″), 6.20 (1H, d, *J*=9.41 Hz, H-3), 6.53 (1H, s, H-8), 6,57 (1H, d, *J*=8.0 Hz, H-12′), 6.79 (1H, s, H-5′), 6. 9 7 (1H, d, *J*=8.3 Hz, H-5′), 7.19 (1H, d, *J*=7.8, H-11′), 7.53 (1H, d, *J*=8.3 Hz, H-6′), 7.93 (1H, d, *J*=9.7 Hz, H-4); ^{13}C NMR (CD_3OD): δ 21.4 (CH_3-C=O-), 28.7 (C-5″), 30.1 (C-3″), 35.5 (C-2″), 62.3 (C-1″), 71.1 (C-4″), 110.4 (C-8), 110.5 (C-4a′), 113.2 (C-4a), 114.1 (C-3), 120.4 (C-5), 120.9 (C-6′), 121.5 (C-6), 121.7 (C-3′), 124.1 (C-12′), 127.1 (C-10′), 125.1 (C-5′), 127.1 (C-10′), 130.5 (C-11′), 139.5 (C-8a′), 140.5 (C-4′), 143.8 (C-8′), 144.3 (C-7′), 145.1 (C-4), 152.1 (C-9′), 152.2 (C-8a), 152.9 (C-7), 159.2 (C-2′),161.5 (C-2),168.9 (CH_3C=O); HRFABMS: *m/z* 548.1368 (Calcd. for $C_{29}H_{24}O_{10}$, 548.1369).

Compound **2**: colourless prisms from $CHCl_3$; m.p. 105-07°C; $[\alpha]_D^{20}$+127° (*c* 0.2, $CHCl_3$); UV-Vis: nm (logε) 210 (3.77); IR (KBr): 3610 (OH), 1750 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 0.41 (2H, m, H-1, H-5), 0.97 (6H, d, *J*=6.3 Hz, H-4′, H-5′); 1.05 (3H, s, H-10), 1.16 (3H, s, H-14), 2.17 (1H, m, H-3′), 2.24 (2H, d, *J*=7.1 Hz, H-2′), 3.32 (1H, m, H-7), 3.77 (1H, m, H-4), 4.65 (1H, ddd, *J*=11.71, 8.11, 6.35 Hz, H-8), 5.45 (1H, d, *J*=3.01 Hz, H-13), 6.11 (1H, d, *J*=2.99 Hz, H-13); ^{13}C NMR ($CDCl_3$): δ 9.3 (C-1), 18.7 (C-14), 19.5 (C-15), 21.5 (C-4′), 21.5 (C-5′), 25.1 (C-3′), 27.2 (C-5), 28.5 (C-2), 30.3 (C-10), 30.5 (C-3), 36.5 (C-9), 40.1 (C-3′), 42.1 (C-2′), 49.7 (C-7), 65.7 (C-4), 66.9 (C-8), 122.7 (C-11), 148.8 (C-13); HREIMS: *m/z* 334.2141 (calcd for $C_{20}H_{30}O_4$, 334.2144).

Compound **3**: colourless prisms from $CHCl_3$; m.p. 132-34°C; $[\alpha]_D^{20}$+187.5° (*c* 0.2, $CHCl_3$); UV-Vis: (logε) 215 (4.11); IR (KBr): 3558 (OH), 1765 (C=O), 1722 (C=O), 1665 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 0.99 (3H, s, H-15), 1.11 (3H, d, *J*=6.2 Hz, H-14), 2.95 (1H, m, H-7),.3.82 (3H, s, -OCH_3), 3.86 (3H, s, -OCH_3), 4.09 (1H, dd, *J*=3.0, 9.0 Hz, H-6), 4.38 (1H, ddd, *J*=11.1, 10.2, 3.1 Hz, H-8), 5.77 (1H, d, *J*=3.1 Hz, H-13), 5.92 (1H, d, *J*=4.4 Hz, H-3′), 6.07 (1H, d, *J*=4.4 Hz, H-5′), 6.21 (1H, d, *J*=2.9 Hz, H-13); ^{13}C NMR ($CDCl_3$) δ 19.8 (C-14), 20.3 (C-15), 24.5 (C-3), 37.7 (C-2), 44.1 (C-9), 45.8 (C-1), 49.5 (C-10), 52.0 (C-7), 56.4 (-OCH_3), 57.1 (-OCH_3), 57.7 (C-5), 74.1 (C-6), 76.5 (C-8), 105.7 (C-5′), 107.8 (C-3′), 110.3 (C-1′), 120.3 (C-11), 140.3 (C-13), 149.1 (C-6′), 150.1 (C-4′), 150.7 (C-2′), 166.1 (C-7′), 167.3 (C-12), 221.5 (C-4); HRFABMS: *m/z* 444.1779 (calcd for $C_{24}H_{28}O_8$, 444.1784).

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