Three New Sesquiterpenoids from *Chrysanthemum indicum* L.

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Phytochemical investigation of the flowers of *Chrysanthemum indicum* L. led to the isolation of three new sesquiterpenoids, indicumolide A (**1**), indicumolide B (**2**), and indicumolide C (**3**). Their structures were elucidated by various spectroscopic methods.

Introduction. – The flower of *Chrysanthemum indicum* L., called *yejuhua* in China, is not only a traditional Chinese medicine, but also a special tea to drink because of its savour and effect. In China, it is used as analgesic and antipyretic to treat inflammations, headache, and vertigo, *etc.* [1]. Previous phytochemical investigations of the EtOH extract of the plant resulted in the isolation of a series of terpenoids, flavonoids, and acids [2–11]. In the course of our studies on the constituents of the H_2O extract of the flowers of *C. indicum* L., two new guaiane-type sesquiterpenoids, indicumolide A (**1**) and indicumolide B (**2**), and a new cadinane-type sesquiterpenoid, indicumolide C (**3**), were isolated from this plant. Their structures were elucidated by various spectroscopic methods. Furthermore, the cytotoxic activities of compounds **1**–**3** were evaluated against five human tumor cell lines, respectively.

Results and Discussion. – Three volumes of 95% EtOH were added to the condensed H_2O extract ($d = 1.16$; 10 l) of *C. indicum* L. The resulting precipitate was removed and the supernatant solution was concentrated to give a residue, which was purified by repeated column chromatography to afford the three new sesquiterpenoids **1**–**3** (*Fig. 1*).

Compound **1** was obtained as colorless crystals. The molecular formula $C_{20}H_{28}O_6$ of **1** was determined on the basis of the HR-ESI-MS, which gave a *pseudo*-molecular-ion peak at *m/z* 387.1775 ($[M+Na]^+$, calc. 387.1784) and indicated seven degrees of unsaturation. The IR spectrum of **1** showed the presence of OH groups (3566 cm^{-1}), CO groups (1755, 1715 cm^{-1}), and C=C bonds (1651 cm^{-1}). In the ^{1}H-NMR spectrum of **1** (*Table*), one olefinic H-atom signal (δ(H) 6.15 (*q*, $J = 7.0$)), three O-bearing CH groups (δ(H) 4.91 (br. *t*, $J = 10.5$), 4.06 (*t*, $J = 9.5$), and 3.87 (*d*, $J = 4.0$)), and five Me groups (δ(H) 2.01 (*d*, $J = 6.5$), 1.90 (*s*), 1.78 (*s*), 1.57 (*s*), 1.30 (*d*, $J = 7.0$)) were observed. The ^{13}C-NMR spectrum of **1** (*Table*) showed 20 C-atom signals, including two CO groups (δ(C) 177.6 and δ(C) 166.7), four C=C bond C-atoms (δ(C) 139.7, 137.1, 127.2, and 126.5), four O-bearing C-atoms (δ(C) 83.2, 79.2, 78.9, and 71.0), five

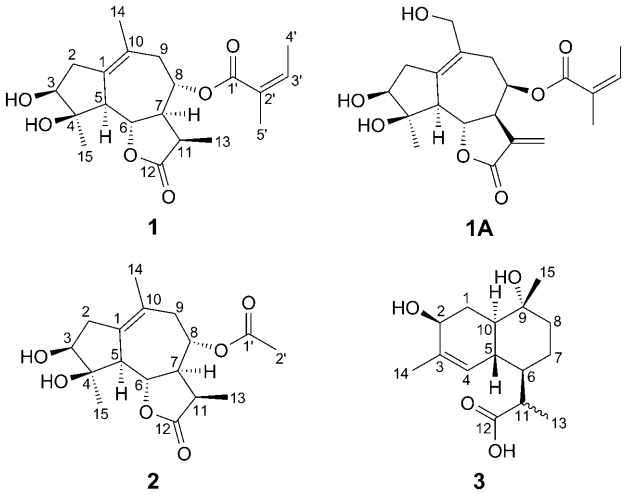


Fig. 1. *Structures of Compounds* **1**, **2**, *and* **3**

Me (δ(C) 15.0, 15.8, 20.5, 23.5, and 23.9), two CH_2 (δ(C) 38.9 and 42.7), and three CH groups (δ(C) 52.8, 58.4, and 40.7). Four degrees of unsaturation were attributed to two CO groups and two pairs of C=C bonds; the remaining three degrees of unsaturation indicated that **1** had a tricyclic ring skeleton. Based on the above evidence and the fact that some sesquiterpenoids, especially *Yejuhua* lactone and its analogs were isolated from this plant [11], compound **1** was suggested to be a tricyclic sesquiterpenoid with guaiane-type skeleton.

Comparison of the ^{13}C-NMR data of **1** with those of eupachinilide A (**1A**) [12], showed that two Me groups Me(13)[1]) (δ(C) 15.0) and Me(14) (δ(C) 23.5) and a CH group C(11) (δ(C) 40.7) are present in the sesquiterpene skeleton of **1**, instead of the signals of one C=C bond and a HO−CH_2 group in eupachinilide A. This was verified by HMBC correlations of Me(13) (δ(H) 1.30, *d*, *J* = 7.0)/C(7) (δ(C) 58.4), C(11) (δ(C) 40.7), and C(12) (δ(C) 177.6); and Me(14) (δ(H) 1.78, *s*)/C(1) (δ(C) 137.1), C(9) (δ(C) 42.7), and C(10) (δ(C) 126.5). Additionally, the HMBC correlations of H−C(5′) with C(1′), C(2′), and C(3′), of Me(4′) with C(1′), C(2′), and C(3′), and of H−C(3′) with C(1′), revealed the presence of a 2′-methylbut-2′-enoyl moiety in **1**, and its (*Z*)-configuration was determined by the correlation of Me(5′) with H−C(3′) in the ROESY experiment. The esterification position was fixed at C(8) through the HMBC correlation of H−C(8) with C(1′), C(10), and C(11) (*Fig. 2*).

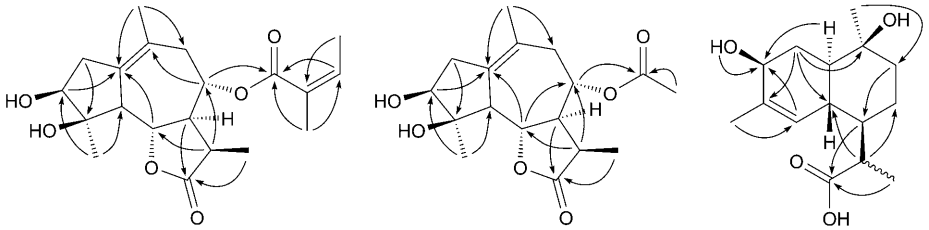
The relative configuration of **1** was established by a ROESY experiment, in which the correlations of H−C(5) with H−C(7), H−C(11), and Me(15), of H−C(3) with Me(15), and of H−C(6) with H−C(8) were observed. Because H−C(5) of the guaiane-type sesquiterpenoid is defined in the α-configuration, and there was no correlation between H−C(5) and H−C(6), the H−C(3), H−C(5), H−C(7), H−C(11), and Me(15) were deduced to be α-oriented, while H−C(6) and H−C(8)

[1]) Arbitrary numbering. For systematic names, see *Exper. Part*.

Table. *^1H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data for Compounds* **1**, **2**, *and* **3**[i])[a])

Position	**1**[b])		**2**[b])		**3**[c])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		137.1		137.1	1.98 (*d*, *J* = 12.5, H_α), 1.09 (*ddd*, *J* = 12.5, H_β)	31.6
2	2.84 (br. *d*, *J* = 16.5), 2.37 (br. *d*, *J* = 16.5)	38.9	2.82 (br. *d*, *J* = 16.5), 2.36 (br. *d*, *J* = 16.5)	39.0	3.73–3.75 (*m*)	66.0
3	3.87 (*d*, *J* = 4.0)	78.9	3.86 (*d*, *J* = 4.5)	78.8		135.6
4		83.2		83.2	5.54 (br. *s*)	125.3
5	2.73 (*d*, *J* = 10.0)	52.8	2.71 (*d*, *J* = 10.0)	52.8	1.66–1.69 (*m*)	39.9
6	4.06 (*t*, *J* = 9.5)	79.2	4.02 (*t*, *J* = 10.0)	79.0	1.20–1.23 (*m*)	44.4
7	2.55–2.58 (*m*)	58.4	2.51–2.54 (*m*)	58.1	1.61 (br. *d*, *J* = 11.5), 1.25 (br. *d*, *J* = 12.5)	25.2
8	4.91 (br. *t*, *J* = 10.5)	71.0	4.78 (*td*, *J* = 11.0, 2.0)	71.6	1.58–1.61 (*m*), 1.36 (*d*, *J* = 12.5)	41.8
9	2.54 (br. *d*, *J* = 14.0), 2.23 (br. *d*, *J* = 14.0)	42.7	2.45–2.49 (*m*), 2.19–2.21 (*m*)	41.8		69.5
10		126.5		126.4	1.39 (*d*, *J* = 11.5)	42.9
11	2.28 (br. *q*, *J* = 11.0)	40.7	2.23 (br. *q*, *J* = 11.0)	40.7	2.74 (br. *q*, *J* = 7.0)	38.7
12		177.6		177.5		176.2
13	1.30 (*d*, *J* = 7.0)	15.0	1.32 (*d*, *J* = 7.0)	15.1	1.01 (*d*, *J* = 7.0)	14.3
14	1.78 (*s*)	23.5	1.77 (*s*)	23.5	1.66 (*s*)	21.2
15	1.57 (*s*)	23.9	1.57 (*s*)	24.0	0.89 (*s*)	20.7
1′		166.7		169.9		
2′		127.2	2.09 (*s*)	21.2		
3′	6.15 (*q*, *J* = 7.0)	139.7				
4′	2.01 (*d*, *J* = 6.5)	15.8				
5′	1.90 (*s*)	20.5				
2-OH					4.51 (*d*, *J* = 5.5)	
9-OH					4.08 (*s*)	
12-OH					12.03 (*s*)	

[a]) Assignments based on HMBC. [b]) Measured in $CDCl_3$. [c]) Measured in (D_6)DMSO.



1 **2** **3**

Fig. 2. *Selected HMBC* (H → C) *correlations for* **1**, **2**, *and* **3**

were assigned to β-orientation. The configuration of H–C(8) in **1** is different from eupachinilide A, consistent with the fact that the coupling constant of H–C(8) in **1** was 10.5 Hz, but only 5.9 Hz in eupachinilide A. In order to clearly show the correlations

disclosed by the ROESY experiment, a 3D structure of **1** (*Fig. 3*) was generated by computer modeling using the program Chem3D pro 11.0. Combining all information above, the structure of compound **1** was elucidated as 8α-(angelyloxy)-3β,4β-dihydroxy-5αH,6βH,7αH,11αH-guai-1(10)-en-12,6-olide, and is was named indicumolide A.

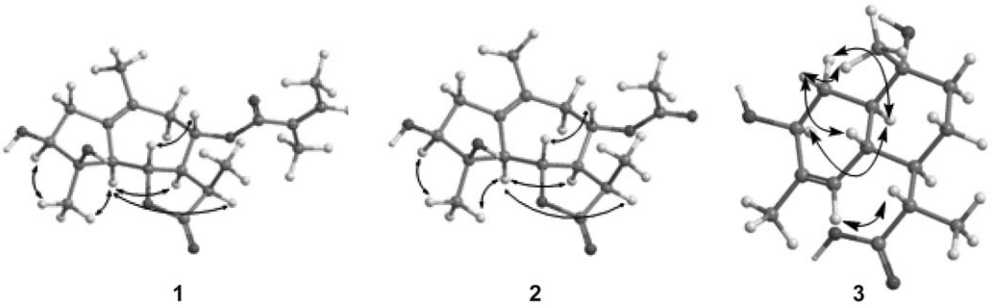


Fig. 3. *Selected ROESY* (H$\leftrightarrow$H) *correlations for* **1**, **2**, *and* **3**

Compound **2** was also obtained as colorless crystals and determined to have a molecular formula $C_{17}H_{24}O_6$ by HR-ESI-MS, which gave a *pseudo*-molecular-ion peak at *m*/*z* 347.1472 ($[M+Na]^+$, calc. 347.1471). The IR spectrum showed the presence of OH (3489 cm^{-1}) and CO groups (1774, 1718 cm^{-1}), and a C=C bond (1675 cm^{-1}). The NMR spectroscopic data of **2** (*Table*) resembled those of **1** except for the missing resonances assigned to the 2′-methylbut-2′-enoyl moiety in **1**, showing an AcO group instead, which showed the resonance signals at Me(2′)[1]) (δ(H) 2.09, *s*) and a CO C-atom C(1′) (δ(C) 169.9). The HMBC correlation of H−C(8)/C(1′) indicated the AcO position was also at C(8) (*Fig. 2*). Furthermore, the similar ROESY correlations and the optical rotation value ($[\alpha]_D^{20} = -24.5$ ($c = 0.07$, $CHCl_3$)) suggested that **2** had the same relative configuration as **1** (*Fig. 3*). So, the compound **2** was elucidated as 8α-acetoxy-3β,4β-dihydroxy-5αH,6βH,7αH,11αH-guai-1(10)-en-12,6-olide, and named indicumolide B.

Compound **3** was isolated as a white power, and its HR-ESI-MS gave a *pseudo*-molecular ion-peak at *m*/*z* 267.1597 ($[M-H]^-$, calc. 267.1596), in agreement with the formula $C_{15}H_{24}O_4$ with four degrees of unsaturation. The IR spectrum showed absorptions due to OH (3436 cm^{-1}) and CO groups (1691 cm^{-1}), and a C=CH group (3232 cm^{-1}). In the ^{1}H-NMR (*Table*), three Me groups ((δ(H) 0.89 (*s*), 1.01 (*d*, J = 7.0), 1.66 (*s*)) and one C=C bond signal (δ(H) 5.54 (br. *s*, 1 H)) were present. The ^{13}C-NMR (*Table*) and HSQC of **3** indicated 15 C-atom signals, including three Me groups (δ(C) 14.3, 20.7, and 21.2), three CH_2 groups (δ(C) 25.2, 31.6, and 41.8), four CH groups (δ(C) 38.7, 39.9, 42.9, 44.4), one O-bearing CH group (δ(C) 66.0), one sp^3 O-bearing quaternary C-atom (δ(C) 69.5), a C=C bond (δ(C) 135.6 and 125.3), and a CO group (δ(C) 176.2). Comparison of the NMR data of sesquiterpene acids from *Leucanthemopsis pulverulenta* [13], with the NMR data of **3** disclosed similarities and suggested that **3** was a cadinane-type sesquiterpene. In the HMBC spectrum (*Fig. 2*), the correlations of Me(13)/C(12)[1]) and H−C(11)/C(12) suggested the presence of a −CH(Me)−COOH moiety, and the attachment position was deduced to be C(6) due

to the correlations of H–C(6)/C(12) and Me(13)/C(6). The correlations of Me(15)/C(10) and C(8), and of HO–C(9)/C(15) confirmed that the Me group (δ(C) 20.7) and the OH group are linked to the O-bearing quaternary C-atom (C(9)). In addition, the correlations of H–C(1)/C(3), C(5), and C(10); H–C(4)/C(2), C(5), C(10), and C(6); and H–C(2)/C(3) and C(4) indicated that the other OH group is linked to C(2), and that the C=C bond is located between C(3) and C(4).

In the ROESY experiment for **3** (*Fig. 3*), correlations of H–C(10)/H_α–C(1) and H–C(2); H_β–C(1)/Me(15) and H–C(5) were observed. Taking for granted that H–C(5) has β-configuration, H–C(10) was determined to have α-configuration due to the fact that there was no correlation between H–C(5) and H–C(10). Consequently, H_α–C(1) and H–C(2) have α-configuration, Me(15) has β-configuration. Additionally, a strong correlation between H–C(4) and H–C(11) in the NOESY experiment suggested the H–C(6) has α-configuration. Thus, the structure of **3** was elucidated except the relative configuration at C(11), as 2β,9α-dihydroxycadin-3(4)-en-12-acid, and named indicumolide C.

Cytotoxic activities of compounds **1**–**3** were evaluated against HCT-8, A549, Bel-7402, BGC823, and A2780 using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. Unfortunately, all compounds exhibited were inactive ($IC_{50} > 10$ μM).

Experimental Part

General Experimental Procedures. Silica gel (SiO_2; 100–200, 200–300 mesh). TLC: silica gel *GF-254* (*Branch of Qingdao Haiyang Chemical Plant*, P. R. China). M.p.: *Reichert Nr-229* micromelting point apparatus; uncorrected. Optical rotations: *Perkin-Elmer 34/LC* polarimeter. IR Spectra: *IMPACT 400* (KBr) spectrometer. ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), ROESY, HMQC, and HMBC Spectra: *INOVA-500* spectrometer with tetramethylsilane (TMS) as internal standard; values given in ppm (δ). ESI-MS: *Agilent 1100* series LC/MSD Trap mass spectrometer (SL). HR-ESI-MS: *ACCU TOF CS* mass spectrometer (*CJMS-T100CS*).

Plant Material. The flowers of *Chrysanthemum indicum* L. were collected from Shanxi province of P. R. China in July 2006. The plant material was identified by Professor *Lin Ma* (Institute of Materia Medica, Peking Union Medical College, and Chinese Academy of Medical Science, P. R. China). A voucher specimen has been deposited in the Herbarium of the Department of Medicinal plants, Institute of Materia Medica Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, P. R. China.

Extraction and Isolation. The dried flowers of *Chrysanthemum indicum* L. (5.0 kg) were exhaustively extracted two times with distilled H_2O. The extract was then concentrated into small volumes (10.0 l; d = 1.16) under reduced pressure, and 30.0 l of 95% EtOH were added. The resulting precipitate was removed and the supernatant soln. was concentrated under reduced pressure to give a residue (350 g). Part of the residue (250 g) was chromatographed over SiO_2 eluting with $CHCl_3$/MeOH (in gradient) to yield nine fractions (*Fr. 1–9*). *Fr. 2* was chromatographed over a SiO_2 column and eluted with petroleum ether (PE)/acetone (in gradient) to give four subfractions (*Frs. 2.1–2.4*). From *Fr. 2.3* (PE/acetone 8:1), compound **1** (20.0 mg) was crystallized from acetone as a white powder. From *Fr. 3* ($CHCl_3$/MeOH 50:1), compound **2** (80.0 mg) was crystallized from $CHCl_3$ to yield a white powder. *Fr. 4* was chromatographed over a SiO_2 column and eluted with PE/acetone (in gradient) to give six subfractions (*Frs. 4.1–4.6*); from *Fr. 4.5* (PE/acetone 5:1), compound **3** (16.0 mg) was crystallized from acetone as a white powder.

Cytotoxicity Experiments. Cytotoxicity against human tumor cells was measured in a 5-day MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide) test for HCT-8, A549, Bel-7402,

BGC823, and A2780. Briefly, 1.5×10^3 cells/100 µl were seeded in 96-well microplates and preincubated for 72 h to allow cell attachment. This medium was then aspirated and 100 µl of fresh medium containing various concentrations of compounds **1**–**3** were added to the cultures. The cells were incubated with each sample for 5 d. Cell survival was evaluated by adding 50 µl of MTT reagent (5 mg MTT/ml in RPMI 1640 medium) to each well. After 4 h reincubation at 37°, 100 µl DMSO was added to dissolve the precipitate of reduced MTT. Microplates were agitated on a rotation platform at r.t. for 15 min, and the absorbance of the mixtures was determined at 570 nm with a multiwell scanning spectrophotometer.

Indicumolide A (=*8α-(Angelyloxy)-3β,4β-dihydroxy-5α*H,*6β*H,*7α*H,*11α*H-*guai-1(10)-en-12,6-olide*; *(3*R**,3a*R**,4*S**,8*S**,9*R**,9a*S**,9b*S**)-2,3,3a,4,5,7,8,9,9a,9b-Decahydro-8,9-dihydroxy-3,6,9-trimethyl-2-oxoazuleno[4,5-*b*]furan-4-yl (2*Z*)-2-Methylbut-2-enoate*; **1**). Colorless crystals. $[\alpha]_D^{20} = -24.4$ ($c = 0.045$, $CHCl_3$). IR (KBr): 3566, 2978, 2944, 2875, 2856, 1755, 1715, 1651, 1455, 1380, 1230, 1158, 1045, 996, 964, 854. ^{1}H- and ^{13}C-NMR (500/125 MHz, $CDCl_3$): *Table*. ESI-MS (pos.): 387.2 ($[M+Na]^+$), 751.4 ($[2M+Na]^+$). HR-EI-MS: 387.1775 ($[M+Na]^+$, $C_{20}H_{28}NaO_6^+$; calc. 387.1784).

Indicumolide B (=*8α-Acetoxy-3β,4β-dihydroxy-5α*H,*6β*H,*7α*H,*11α*H-*guai-1(10)-en-12,6-olide*; *(3*R**,3a*R**,4*S**,8*S**,9*R**,9a*S**,9b*S**)-2,3,3a,4,5,7,8,9,9a,9b-Decahydro-8,9-dihydroxy-3,6,9-trimethyl-2-oxoazuleno[4,5-*b*]furan-4-yl Acetate*; **2**). Colorless crystals. $[\alpha]_D^{20} = -24.5$ ($c = 0.070$, $CHCl_3$). IR (KBr): 3489, 3384, 3004, 2978, 2935, 2884, 2860, 1774, 1718, 1675, 1452, 1378, 1252, 1142, 1040, 995, 957, 880. ^{1}H- and ^{13}C-NMR (500/125 MHz, $CDCl_3$): *Table*. ESI-MS (pos.): 347 ($[M+Na]^+$), 671 ($[2M+Na]^+$). HR-EI-MS: 347.1472 ($[M+Na]^+$, $C_{17}H_{24}NaO_6^+$; calc. 347.1471).

Indicumolide C (=*2β,9α-Dihydroxycadin-3(4)-en-12-acid*; *2-[(1*R**,4*R**,4a*R**,6*S**,8a*R**)-1,2,3,4,4a,5,6,8a-Octahydro-4,6-dihydroxy-4,7-dimethylnaphthalen-1-yl]propanoic Acid*; **3**). White powder. $[\alpha]_D^{20} = 21.7$ ($c = 0.060$, MeOH). IR (KBr): 3436, 3232, 2923, 2874, 1691, 1583, 1269, 1122, 924, 861, 833, 795. ^{1}H- and ^{13}C-NMR (500/125 MHz, (D_6)DMSO): *Table*. ESI-MS (neg.): 267.2 ($[M-H]^-$). HR-ESI-MS: 267.1597 ($[M-H]^-$, $C_{15}H_{23}O_4^+$; calc. 267.1596).

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