New Monoterpenoids from Fruits of *Gardenia jasminoides* var. *radicans*

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One new lactone, cyclopentanepyrone A (**1**), and two new monoterpenoids, gardeterpenone A (**2**) and jasminoside V (**3**), were isolated from the fruits of *Gardenia jasminoides* var. *radicans*, along with four known monoterpenoids, **4**–**7**, which were isolated from this plant for the first time. The structures of the isolates were elucidated by extensive spectroscopic studies, including UV, IR, 1D- and 2D-NMR, ESI-MS, HR-ESI-MS, and CD experiments.

Introduction. – *Gardenia jasminoides* var. *radicans*, as a variant of *G. jasminoides* J.ELLIS [1], is mainly distributed in some southern provinces of China. At present, there are only a few reports on its chemical constituents [2–6]. Our previous investigations of this plant revealed the presence of 13 iridoids, five flavonoids, four crocins, one monoterpene, five triterpenes, flavonoid glycosides, and five other compounds [4–7]. Our present investigation led to the discovery of three new monoterpenoid compounds, namely one new lactone, cyclopentanepyrone A (**1**), one new monoterpene, gardeterpenone A (**2**), and one monoterpenoid glycoside, jasminoside V (**3**), from the fruits of this plant, as well as four known monoterpenoids, rehmapicrogenin (**4**), 6′-*O*-sinapoyljasminoside A (**5**), jasminoside B (**6**), and jasminoside M (**7**), which are also reported from this plant for the first time (*Fig. 1*).

Results and Discussion. – Compound **1** was obtained as brownish oil. Its HR-ESI-MS exhibited a protonated molecular ion at *m*/*z* 183.1018 ($[M+H]^+$), in agreement with the molecular formula $C_{10}H_{14}O_3$. The UV absorption maximum at 217 nm, and IR absorption bands at 3420, 1760, 1683, and 1025 cm^{-1} suggested that **1** contains a OH and an α,β-unsaturated lactone group. The NMR data (*Table 1*) of **1** showed a C=C bond between C(4) (δ(H) 6.64 (*t*, $J = 3.0$), δ(C) 136.8) and C(5) (δ(C) 131.3); one COO signal at δ(C) 172.3 (C(3)); two Me signals at δ(H) 0.88 (*s*, Me(10))/δ(C) 20.0 (C(10)) and δ(H) 1.07 (*s*, Me(11))/δ(C) 29.8 (C(11)); two CH_2 signals at δ(H) 4.07–3.96 (*m*, H_a–C(1)), 4.52 (*t*, $J = 9.0$, H_b–C(1))/δ(C) 69.8 (C(1)) and δ(H) 1.46 (*dd*, $J = 12.9$, 10.2, H_a–C(7)), 1.88 (*dd*, $J = 13.0$, 6.2, H_b–C(7))/δ(C) 47.5 (C(7)); one CH signal at δ(H) 3.02 (*tt*, $J = 9.5$, 3.8, H–C(9))/δ(C) 47.6 (C(9)), one CH–O signal at δ(H) 4.46–4.35 (*m*, H–C(6))/δ(C) 67.3 (C(6)), and one saturated C_q-atom signal at δ(C) 36.2 (C(8)).

In addition, the presence of a δ-lactone unit was supported by the COO signal at δ(C) 172.3 (C(3)). The HMB correlations of H–C(4) with C(3), C(5), C(6), and C(9), as well as the COSY correlations H–C(6)/CH_2(7) and H–C(9)/CH_2(1) suggested the presence of a –O–CH_2–CH–C(=CH–C(O)–)–CH(OH)–CH_2– moiety (*Fig. 2*). The

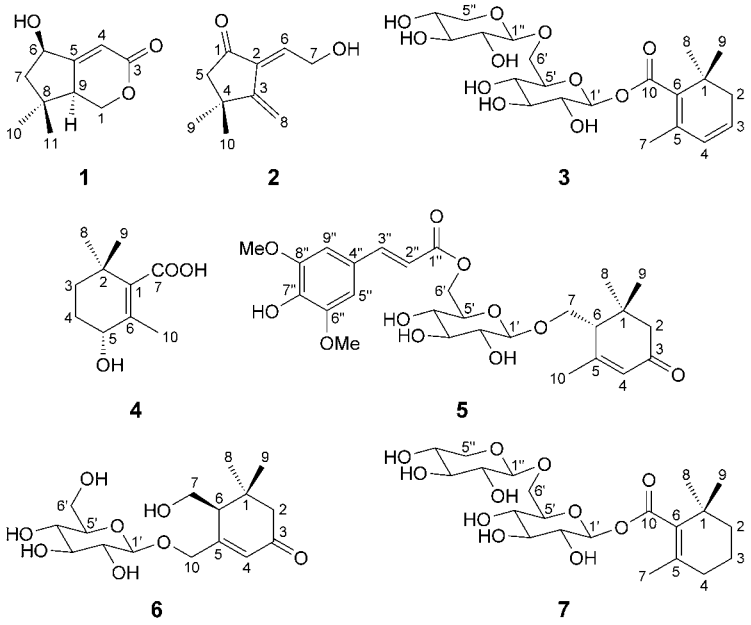

Fig. 1. *Structures of* **1**–**7**

Table 1. *^{1}H- and ^{13}C-NMR Data* (300 and 75 MHz, resp.; in CD_3OD) *of* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	4.07–3.96 (*m*, H_a)[a]), 4.52 (*t*, $J = 9.0$, H_b)	69.8		202.1
2				159.0
3		172.3		150.3
4	6.64 (*t*, $J = 3.0$)	136.8		40.0
5		131.3	2.35 (*s*)	53.1
6	4.46–4.35 (*m*)[a])	67.3	6.18 (*s*)	123.8
7	1.46 (*dd*, $J = 12.9$, 10.2, H_a), 1.88 (*dd*, $J = 12.9$, 6.2, H_b)	47.5	4.41 (*d*, $J = 1.5$)	62.0
8		36.2	5.42 (*s*, H_a), 5.39 (*d*, $J = 1.5$, H_b)	114.1
9	3.02 (*tt*, $J = 9.5$, 3.8)	47.6	1.17 (*s*)	28.6
10	0.88 (*s*)	20.0	1.17 (*s*)	28.6
11	1.07 (*s*)	29.8		

[a]) Signal multiplicity pattern is unclear due to overlapping.

Me(10) and Me(11) groups correlated with C(8), C(7), and C(9) in the HMBC spectrum, indicating that the two Me groups were connected to the saturated C_q-atom C(8). Furthermore, a HMB correlation of H_b–C(1) with C(3) was observed. Taking into account the four degrees of unsaturation, the planar structure of **1** was deduced.

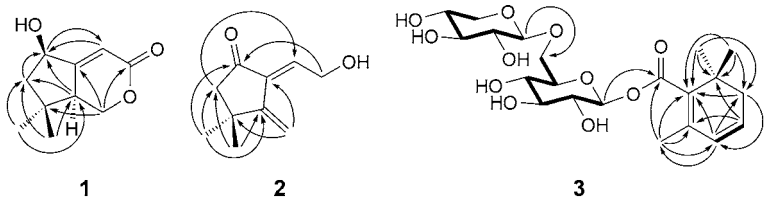

Fig. 2. *Key HMBC* (H → C) *and COSY* (—) *correlations of* **1**–**3**

The ^{1}H- and ^{13}C-NMR signals (*Table 1*) of **1** were completely assigned with the aid of the ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY spectra.

Additionally, the NOE correlation between H–C(6) and H–C(9) suggested that these H-atoms are on the same side of the molecule plane, leading to either (6*R*,9*S*)- or (6*S*,9*R*)-configuration. The absolute configuration of **1** was elucidated by a quantum-chemical circular dichroism (CD) calculation. The CD computation for **1** was performed at the B3LYP/6–31G(d) level. The overall predicted CD curve was compared with the experimental one, which implied a good agreement between the calculated CD curve and the measured one (*Fig. 3*). Therefore, **1** was identified as (5*R*,7a*S*)-5,6,7,7a-tetrahydro-5-hydroxy-7,7-dimethylcyclopenta[*c*]pyran-3(1*H*)-one and named cyclopentanepyrone A.

Compound **2** was obtained as brownish jelly. The molecular formula, $C_{10}H_{14}O_2$, was determined from its HR-ESI-MS, which showed a *quasi*-molecular-ion peak at *m*/*z* 167.1064 ([*M* + H]$^{+}$). The UV maximum absorptions at 204 and 271 nm, and IR absorptions bands at 3414, 1660, 1032, and 913 cm^{-1} indicated that **2** contains OH and C=O groups, and a conjugated diene unit. This was further supported by ^{1}H- and ^{13}C-NMR spectra (*Table 1*). The 1D- and 2D-NMR data of **2** showed two C=C

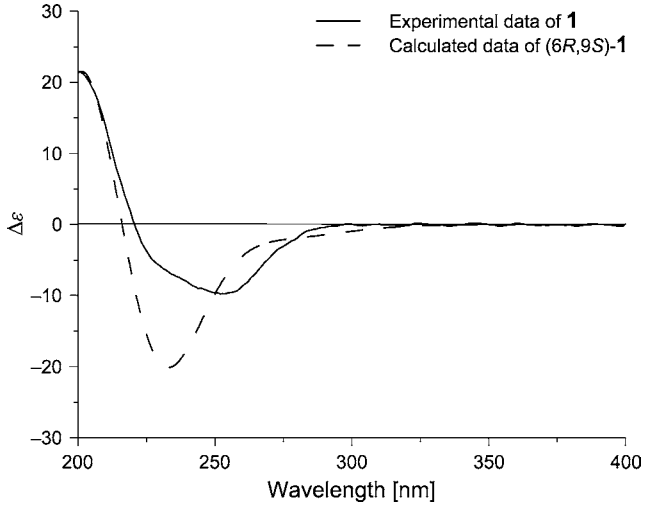

Fig. 3. *CD Spectrum of* **1** *and its computed CD curve*

bonds between C(8) (δ(H) 5.42 (*s*, H_a), 5.39 (*d*, $J = 1.5$, H_b)/δ(C) 114.1) and C(3) (δ(C) 150.3) as well as C(6) (δ(H) 6.18 (*s*)/δ(C) 123.8) and C(2) (δ(C) 159.0), one C=O signal at δ(C) 202.1 (C(1)), two Me signals at δ(H) 1.17 (*s*, Me(9,10))/δ(C) 28.6 (C(9,10)), one CH_2 signal at δ(H) 2.35 (*s*, H–C(5))/δ(C) 53.1 (C(5)); one CH_2O signal at δ(H) 4.41 (*d*, $J = 1.5$, H–C(7))/δ(C) 62.0 (C(7)), and one saturated C_q-atom signal at δ(C) 40.0 (C(4)).

In the HMBC spectrum (*Fig. 2*), the correlations of Me(9) and Me(10) with C(4), C(5), and C(3), suggested that the two Me groups were connected to C(4). The long-range correlations of H_a–C(8), H_b–C(8), and H–C(6) with C(3) and C(2) indicated a conjugated diene unit. Meanwhile, the correlations of CH_2(7) with C(2) and C(6) determined the linkage of the CH_2O group to C(6). Besides, the HMB correlations of H–C(6) with C(1) and of CH_2(5) with C(1) indicated that the C=O group (C(1)) was located between C(5) and C(2). Therefore, **2** was identified as (2*E*)-2-(2-hydroxyethylidene)-4,4-dimethyl-3-methylidenecyclopentanone, and named gardeterpenone A.

Compound **3** was obtained as white jelly. The molecular formula, $C_{21}H_{32}O_{11}$, was deduced from the *pseudo*-molecular-ion peak at *m*/*z* 483.1036 ($[M + Na]^+$) in the positive-ion-mode HR-ESI-MS. The UV maximum peaks at 204 and 277 nm, and the IR absorption bands at 3404, 1713, and 1061 cm^{-1} revealed that **3** contains OH groups, an ester C=O group, and a conjugated diene unit. According to the anomeric H-atom signal at δ(H) 5.60 (*d*, $J = 7.9$, H–C(1′)) and the C-atom signals at δ(C) 95.8 (C(1′)), 74.0 (C(2′)), 78.2 (C(3′)), 71.1 (C(4′)), 78.1 (C(5′)), and 69.6 (C(6′)) (*Table 2*), the presence of a β-D-glucopyranosyl moiety was indicated. Meanwhile, a β-D-xylopyranosyl unit was deduced based on the anomeric H-atom signal at δ(H) 4.35 (*d*, $J = 7.4$, H–C(1″)) and the C-atom signals at δ(C) 105.3 (C(1″)), 75.0 (C(2″)), 77.7 (C(3″)), 71.2 (C(4″)), and 66.9 (C(5″)) [8]. Acid hydrolysis and derivatization of **3** followed by HPLC analysis of the derivatives, determined β-D-glucose and β-D-xylose as monosaccharide residues in the molecule [9]. Except for the signals of two sugar residues, the ^{13}C-NMR and DEPT spectra of **3** exhibited ten C-atom signals, including

Table 2. *^{1}H- and ^{13}C-NMR Data* (300 and 75 MHz, resp.; in CD_3OD) *of* **3**. δ in ppm, *J* in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1		34.6	3′	3.47 (*d*, $J = 2.7$)	78.2
2	2.08–2.22 (*m*)[a])	40.7	4′	3.50 (*s*)	71.1
3	5.97 (*dt*, $J = 9.6, 4.0$)	130.4	5′	3.60 (*dd*, $J = 8.2, 4.9$)	78.1
4	5.90 (*dt*, $J = 9.6, 1.5$)	129.2	6′	4.11 (*dd*, $J = 11.6, 1.7$),	69.6
5		134.9		3.80–3.75 (*m*)[a])	
6		133.8	1″	4.35 (*d*, $J = 7.4$)	105.3
7	1.86 (*s*)	20.2	2″	3.23 (*dd*, $J = 5.3, 3.5$)	75.0
8	1.16 (*s*)	26.6	3″	3.33 (*dd*, $J = 3.1, 1.7$)	77.7
9	1.15 (*s*)	26.3	4″	3.40–3.35 (*m*)[a])	71.2
10		170.1	5″	3.87 (*dd*, $J = 11.4, 5.2$),	66.9
1′	5.60 (*d*, $J = 7.9$)	95.8		3.17 (*d*, $J = 11.4$)	
2′	3.44 (*s*)	74.0			

[a]) Signal multiplicity pattern is unclear due to overlapping.

an ester C=O group (δ(C) 170.1 C(10)), two olefinic C_q-atoms (134.9 (C(5)) and 133.8 (C(6))), two olefinic CH groups (δ(H) 5.97 (*dt*, $J = 9.6, 4.0$, H–C(3))/δ(C) 130.4 (C(3)) and δ(H) 5.90 (*dt*, $J = 9.6, 1.5$, H–C(4))/δ(C) 129.2 (C(4))), a CH_2 group (δ(H) 2.08–2.22 (*m*, CH_2(2))/δ(C) 40.7 (C(2))), a saturated C_q-atom (δ(C) 34.6 (C(1))), together with three Me groups (δ(H) 1.86 (*s*, Me(7))/δ(C) 20.2 (C(7)), δ(H) 1.66 (*s*, Me(8))/δ(C) 26.6 (C(8)), and δ(H) 1.65 (*s*, Me(9))/δ(C) 26.3 (C(9))). Complete and combinational analysis of ^{13}C-NMR, HSQC, and HMBC spectra allowed deducing the constitution of the aglycone moiety as a monoterpene (*Fig. 1*).

The HMB correlation of the H-atom at δ(H) 5.60 (H–C(1′)) with the C=O C-atom at δ(C) 170.1 (C(10)) suggested that the glucopyranosyl moiety is attached to the C=O C-atom of the aglycone residue. Besides, the two sugar moieties formed a β-D-Xyl-(1″ → 6′)-β-D-Glc connection on the basis of the correlation of the H-atom at δ(H) 4.35 (H–C(1″)) with the C-atom at δ(C) 69.6 (C(6′)) in the HMBC spectrum. Therefore, **3** was identified as 1-*O*-[(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)carbonyl]-6-*O*-β-D-xylopyranosyl-β-D-glucopyranose. It is the disaccharide of a monoterpene, named jasminoside V.

In addition, the known compounds were identified as rehmapicrogenin (**4**) [10], 6′-*O*-sinapoyljasminoside A (**5**) [11], jasminoside B (**6**) [11], and jasminoside M (**7**) [8] (*Fig. 1*) by comparing their spectroscopic data with reference values.

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Experimental Part

General. Standard sugars, β-D-glucose and α-L-rhamnose, and reagent L-cysteine methyl ester were purchased from *Adamas-beta Company* (Basel, Switzerland). *o*-Tolyl isothiocyanate was purchased from *Sigma Company* (Santa Clara, CA, USA). All reagents were purchased from *Tianjin Damao Chemical Company* (Tianjin, P. R. China). Thin-layer chromatography (TLC): precoated aluminum silica gel *HSAF254* plates (SiO_2; 1 mm; *Yantan*, P. R. China); spraying reagent, 10% H_2SO_4/EtOH. Column chromatography (CC): SiO_2 (300–400 mesh; *Qingdao Haiyang Chemical Group Corporation*; Qingdao, P. R. China), ODS (50 μm; *YMC*, Tokyo, Japan), and *Sephadex LH-20* (*GE Healthcare*, Sweden). Anal. and semi-prep. reversed-phase HPLC: *Agilent 1200* (*Agilent Technologies*, Santa Clara, CA, USA) with an *XB-C18* column (4.6 × 250 mm, 5 μm; *Welch Materials Inc.*) for analysis and an *Ultimate® XB-C18* column (21.2 × 250 mm, 5 μm; *Welch Materials Inc.*) for purification. Optical rotations: *JASCO P-1020* automatic digital polarimeter (*JASCO International Co., Ltd.*, Tokyo, Japan). UV Spectra: *JASCO V-550* UV/VIS spectrometer (*JASCO International Co., Ltd.*, Tokyo, Japan); λ_{max} (log ε) in nm. CD Spectra: *Chirascan* spectropolarimeter (*Applied Photophysics Ltd.*, U.K.); at r.t. IR Spectra: *JASCO FT/IR-480 plus* FT-IR spectrometer (*JASCO International Co., Ltd.*, Tokyo, Japan); $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker Avance III* 300 MHz spectrometer (300 and 75 MHz, resp.; *Bruker Biospin*, Fällanden, Switzerland); in CD_3OD; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *LCQ Advantage Max* mass spectrometer (*Thermo Finnigan*, San Diego, CA, USA); in *m*/*z*. HR-ESI-MS: *Agilent 6210 LC/MSD TOF* mass spectrometer (*Agilent Technologies*, Santa Clara, CA, USA); in *m*/*z*.

Plant Material. Dried ripe fruits of *G. jasminoides* var. *radicans* were collected from Bozhou Market for Chinese Materia Medica, Anhui Province, P. R. China, in October 2011 and authenticated by *G.-X. Zhou* at the College of Pharmacy, Jinan University. A voucher specimen was deposited with the herbal museum of the college, Guangzhou, P. R. China.

Extraction and Isolation. The dried ripe fruits (7.0 kg) of *G. jasminoides* var. *radicans* were ground into powder, and the powder was heated under reflux with 70% EtOH (3 × 15 l, 3 h each). The extract was concentrated *in vacuo* to yield a dark-brown residue (800 g), which was suspended in 3.0 l of H_2O,

and then partitioned with petroleum ether (PE), AcOEt, and BuOH (3 × 3.0 l, 3 h each), successively. The AcOEt extract (80 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:0 to 0:100) to give nine fractions, *Frs. 1–9*, which were merged according to their TLC pattern. *Fr. 3* (7.2 g) was subjected to CC (SiO_2; PE/acetone 50:1, 25:1, 20:1, 10:1, 5:1, 3:1, 1:1, and 0:1) to furnish **4** (23.1 mg) and ten fractions, *Frs. 3.1–3.10*. Furthermore, *Fr. 3.5* (0.4 g) was separated by prep. HPLC (MeCN/H_2O 15:85) to afford **1** (7.2 mg) and **2** (6.8 mg). Compound **5** (11.2 mg) was purified from *Fr. 3.3* (0.4 g) by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1, isocratic solvent system). Additionally, *Fr. 6* (5.8 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 1000:1, 100:1, 50:1, 30:1, 20:1, 10:1, 8:2, 1:1, and 0:1) to give eight subfractions, *Frs. 6.1–6.8*. *Fr. 6.2* (0.5 g) was separated by prep. HPLC (MeOH/H_2O 50:50) to yield **3** (7.9 mg). *Fr. 6.4* (0.9 g) was further subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1, isocratic solvent system) to yield **6** (4.2 mg) and **7** (18.7 mg).

Cyclopentanepyrone A (=(*5*R,*7a*S)*-5,6,7,7a-Tetrahydro-5-hydroxy-7,7-dimethylcyclopenta[c]pyran-3(1*H*)-one*; **1**). Brown oil. $[\alpha]_D^{25} = -15.8$ ($c = 0.50$, MeOH). UV (MeOH): 217 (4.03). IR (KBr): 3420, 2959, 1760, 1683, 1216, 1156, 1025, 751. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 183 ($[M+H]^+$). HR-ESI-MS: 183.1018 ($[M+H]^+$, $C_{10}H_{15}O_3^+$; calc. 183.1016).

Gardeterpenone A (=(2E)*-2-(2-Hydroxyethylidene)-4,4-dimethyl-3-methylidenecyclopentanone*; **2**). Brown jelly. UV (MeOH): 204 (4.05), 271 (4.04). IR (KBr): 3414, 2966, 2937, 1660, 1286, 1105, 1032, 913, 762. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 167 ($[M+H]^+$). HR-ESI-MS: 167.1064 ($[M+H]^+$, $C_{10}H_{15}O_2^+$; calc. 167.1067).

Jasminoside V (=*1*-O*-[(2,6,6-Trimethylcyclohexa-1,3-dien-1-yl)carbonyl]-6*-O-*β*-D-*xylopyranosyl-β*-D-*glucopyranose*; **3**). White jelly. $[\alpha]_D^{25} = -16.2$ ($c = 0.50$, MeOH). UV (MeOH): 204 (4.02), 277 (4.05). IR (KBr): 3404, 2959, 2921, 1713, 1648, 1458, 1377, 1061. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 483 ($[M+Na]^+$). HR-ESI-MS: 483.1836 ($[M+Na]^+$, $C_{21}H_{32}NaO_{11}^+$; calc. 483.1837).

Acid Hydrolysis of **3** *and Determination of the Absolute Configuration of Sugars.* The absolute configurations of the two monosaccharide residues of **3** were identified by the method of *Tanaka* [9]. Compound **3** (1 mg) was hydrolyzed by heating in 2M HCl for 4 h at 70°. After drying *in vacuo*, the residue was dissolved in H_2O and extracted with AcOEt. Then, the aq. layer was collected. After drying under reduced pressure, the residue was dissolved in pyridine (1 ml) containing L-cysteine methyl ester hydrochloride (1 mg) and heated at 60° for 1 h. A soln. (0.1 ml) of *o*-tolylisothiocyanate (0.5 mg) in pyridine was added to the mixture, which was heated at 60° for 1 h. Then, the mixture was directly analyzed by reversed-phase HPLC (*XB-C18*) with isocratic elution of 25% MeCN containing 0.1% AcOH for 60 min; flow rate, 1 ml min^{-1}; UV detector (254 nm). The peaks at t_R 17.34 and 19.52 min coincided with the corresponding derivatives of *β*-D-glucose and *β*-D-xylose.

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