FULL PAPER

Structure Revision of Caesalpinistas A and B and Isolation of a New Furanoditerpenoid from the Cotyledons of *Caesalpinia decapetala* var. *japonica*

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A known cassane-type furanoditerpenoid, caesalpinista B (**1**), and a new diterpenoid, deoxycaesaljaponin A (**2**), were isolated from the cotyledons of *Caesalpinia decapetala* var. *japonica*. The previously reported configurational assignments of **1** and the related diterpenoid caesalpinista A (**3**) were revised on the basis of X-ray crystallography and chemical conversion. The structure of **2** was elucidated by spectroscopic data and chemical conversion into **3**.

Introduction. – Cassane diterpenoids are interesting because of their structural diversity as well as their broad spectrum of biological activities [1]. As part of our continued search for biologically active compounds from plants [2][3], we have isolated two new cassane-type furanoditerpenoids, caesaljaponins A and B [4], from the MeOH extract of seeds of *Caesalpinia decapetala* var. *japonica*; their structures have been elucidated. On further surveying related compounds from this plant, a known cassane-type furanoditerpenoid, caesalpinista B (**1**), and a new furanoditerpenoid, designated deoxycaesaljaponin A (**2**), were isolated.

In 2009, *Yang* and co-workers have reported the cassane-type furanoditerpenoids caesalpinista B (**1′**) along with caesalpinista A (**3′**) from the MeOH extract of the seeds of *Caesalpinia crista*, and their relative configurations have been proposed on the basis of 1D- and 2D-NMR analysis [5]. In particular, the ROESY correlation between Me(19) and H–C(5) was utilized for the assignment of the α-orientation of Me(19). However, the Me(19) signal partially overlapped with the signals of CH_2(2), CH_2(3), CH_2(7), and H–C(9) in the ^{1}H-NMR spectrum, suggesting the need to re-examine the configurational assignments of **1′** and **3′**. This study describes the structure revisions of **1′** and **3′** as well as the isolation and structure elucidation of **2**.

Results and Discussion. – The AcOEt-soluble portion of the MeOH extract of the cotyledons of *C. decapetala* var. *japonica* was subjected to column chromatography (SiO_2; gradient mixtures of AcOEt/hexane) furnishing **1** and **2**.

Compound **1** was obtained as colorless crystals with a molecular formula of $C_{23}H_{32}O_6$, which was established on the basis of HR-ESI-FT-MS data (*m*/*z* 427.2088 ([*M* + Na]$^+$; calc. 427.2091)). Melting point, as well as IR and NMR data (*Table*), were in good agreement with those previously reported for caesalpinista B (**1′**) [5]. These findings indicated that **1** is identical to caesalpinista B. However, the ^{13}C-NMR chemical shift for Me(19) of **1** (δ(C) 18.6) is typical for a β-oriented Me group (18 – 20) [4][6] and not for an α-oriented one (28 – 29) [7 – 9]. These findings suggested that it is necessary to re-examine the configurational assignment as reported for **1′** [5]. As the ^{1}H-NMR spectrum of **1** recorded in $CDCl_3$ showed partially overlapped signals, 1D-difference NOE experiments were performed in C_6D_6. Irradiation of H_a–C(20) enhanced Me(19) (6.5%), indicating that Me(19) is axial and β-oriented. Finally, the structure and the absolute configuration of **1** were unambiguously confirmed by X-ray crystallography using the anomalous scattering of CuK_α radiation, with the *Flack* parameter [10] being refined to 0.07(3) (*Fig. 1*). As a result, the originally reported

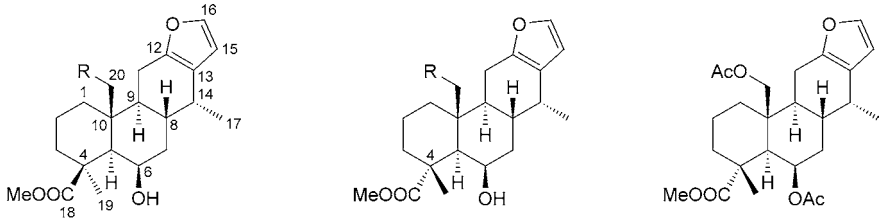

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Table. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; in $CDCl_3$) *of* **1** – **3**

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.00 (*td*, *J* = 13.4, 3.1, H_α), 2.43 (br. *d*, *J* = 13.4, H_β)	34.9	1.00 (*td*, *J* = 13.4, 3.4, H_α), 2.48 (br. *d*, *J* = 13.4, H_β)	34.6	1.14 (*td*, *J* = 13.4, 4.3, H_α), 1.95 (*d*, *J* = 13.4, H_β)	39.4
2	1.56 – 1.63 (*m*, H_α), 1.45 (*qt*, *J* = 13.4, 3.1, H_β)	18.7	1.58 (br. *d*, *J* = 13.4, H_α), 1.46 (br. *q*, *J* = 13.4, H_β)	18.5	1.55 – 1.65 (*m*, H_α), 1.46 (*qt*, *J* = 13.4, 3.1, H_β)	18.6
3	1.79 (*td*, *J* = 13.4, 4.0, H_α), 1.60 (br. *d*, *J* = 13.4, H_β)	38.4	1.77 (*td*, *J* = 13.4, 4.0, H_α), 1.62 (br. *d*, *J* = 13.4, H_β)	38.4	1.81 (*td*, *J* = 13.4, 4.0, H_α), 1.61 (br. *d*, *J* = 13.4, H_β)	38.4
4		48.3		47.7		48.4
5	2.00 (br. *s*)	51.2	2.15 (br. *s*)	49.8	2.03 (br. *s*)	51.1
6	3.96 (br. *s*)	69.4	4.97 (br. *s*)	71.6	3.92 (br. *s*)	69.1
7	1.55 – 1.72 (*m*)	40.1	1.61 (*td*, *J* = 14.3, 2.7, H_α), 1.87 (*dt*, *J* = 14.3, 3.7, H_β)	35.4	1.56 – 1.64 (*m*, H_α), 1.67 (*ddd*, *J* = 14.0, 5.2, 3.1, H_β)	39.4
8	2.18 – 2.26 (*m*)	31.3	2.00 – 2.11 (*m*)	31.7	2.59 (*tt*, *J* = 12.5, 5.2)	32.5
9	1.59 – 1.69 (*m*)	45.5	1.69 (*td*, *J* = 11.0, 6.1)	45.3	1.50 – 1.62 (*m*)	45.2
10		41.1		41.4		41.0
11	2.74 (*dd*, *J* = 16.8, 6.4, H_α), 2.52 (*dd*, *J* = 16.8, 10.7, H_β)	23.0	2.76 (*dd*, *J* = 17.1, 6.1, H_α), 2.55 (*dd*, *J* = 17.1, 11.0, H_β)	23.0	2.69 (*dd*, *J* = 15.9, 6.1, H_α), 2.76 (*dd*, *J* = 15.9, 10.7, H_β)	22.3
12		149.3		149.0		149.3
13		121.9		121.8		122.1
14	2.63 (*qd*, *J* = 7.0, 5.2)	31.1	2.61 (*qd*, *J* = 7.0, 5.2)	30.9	2.51 (*qd*, *J* = 7.0, 5.2)	31.2
15	6.19 (*d*, *J* = 1.8)	109.5	6.18 (*d*, *J* = 1.8)	109.5	6.17 (*d*, *J* = 1.8)	109.6
16	7.22 (*d*, *J* = 1.8)	140.4	7.21 (*d*, *J* = 1.8)	140.5	7.21 (*d*, *J* = 1.8)	140.3
17	0.97 (*d*, *J* = 7.0)	17.8	0.97 (*d*, *J* = 7.0)	17.7	0.93 (*d*, *J* = 7.0)	17.0
18		179.2		178.5		179.3
19	1.60 (*s*)	18.6	1.39 (*s*)	18.1	1.58 (*s*)	18.6
20	4.95 (*d*, *J* = 13.7, H_a), 4.28 (*d*, *J* = 13.7, H_b)	64.3	4.97 (*d*, *J* = 13.4, H_a), 4.21 (*d*, *J* = 13.4, H_b)	63.5	4.28 (*d*, *J* = 12.8, H_a), 3.63 (*d*, *J* = 12.8, H_b)	64.5
MeO	3.70 (*s*)	52.2	3.71 (*s*)	52.0	3.71 (*s*)	52.2
6-AcO			2.07 (*s*)	21.2		
				170.0		
20-AcO	2.07 (*s*)	21.2	2.08 (*s*)	21.7		
		170.9		170.9		

structure of caesalpinista B should be revised from **1′** to **1**.

Caesalpinista B (**1**) was hydrolyzed by K_2CO_3/MeOH, furnishing the deacetyl derivative **3**. IR, ^{1}H- and ^{13}C-NMR, and HR-ESI-MS data of **3** were identical to those of caesalpinista A (**3′**) reported previously [5]. These results indicate that **3** is identical to caesalpinista A, and the originally reported structure of caesalpinista A should be revised from **3′** to **3**.

Deoxycaesaljaponin A (**2**) was obtained as colorless oil, and the molecular formula was established on the basis of positive-ion mode HR-ESI-FT-MS data (*m*/*z* 469.2195 ($[M+Na]^+$; calc. 469.2197)) to be $C_{25}H_{34}O_7$, which differs from that of caesalpinista B (**1**) by 42 amu (C_2H_2O). This difference corresponds to the addition of an Ac group. The hydrolysis of **2** was conducted in a manner similar to that for **1**, furnishing a product. The specific optical rotation, UV, electronic circular dichroism (ECD), IR, ^{1}H- and ^{13}C-NMR, and HR-ESI-FT-MS data of the product were identical to those of **3**. These results suggested **2** to be an acetyl analog of **1**. This assumption was supported by the NMR data of **2** (*Table*), which were very similar to those of caesalpinista B (**1**), except for the presence of an additional Ac group in **2**. The significant downfield shift (+1.0) of H–C(6) in **2** indicated that the additional Ac group is linked to the O-atom at C(6). As the ^{1}H-NMR spectrum of **2** recorded in $CDCl_3$ showed partially overlapped signals, extensive NMR recordings were also performed in C_6D_6. The ^{1}H,^{1}H-COSY and HMB correlations shown in *Fig. 2* support the proposed structure of **2**. The relative configuration of **2** was confirmed by analyses of coupling constants, NOESY data, and 1D-difference NOE experiments (*Fig. 3*). In the ^{1}H-NMR spectrum of **2**, the signal of H–C(6) was a broad *singlet*, indicating that H–C(6) and 6-AcO are in equatorial and axial positions, respectively. The *trans-anti-trans* system of the three six-membered rings and the orientations of H–C(5), H–C(8), H–C(9), and CH_2(20) in **2** were consistent with those of cassane-type diterpenes reported in the literature [1][11 – 13]. The absolute configuration of **2** was also supported by comparison of the experimental ECD spectrum of **2** with that calculated using the time-dependent density-functional theory method. Deoxycaesaljaponin A (**2**) exhibited a positive *Cotton* effect at 218 nm ($\Delta\varepsilon$ +2.57) in the ECD spectrum, attributed to the $\pi \rightarrow \pi^*$ transition of the furan chromophore. The observed ECD spectrum of **2** was in good agreement with that calculated for the model with an α-oriented Me(17) (*Fig. 4*). Thus, deoxycaesaljaponin A

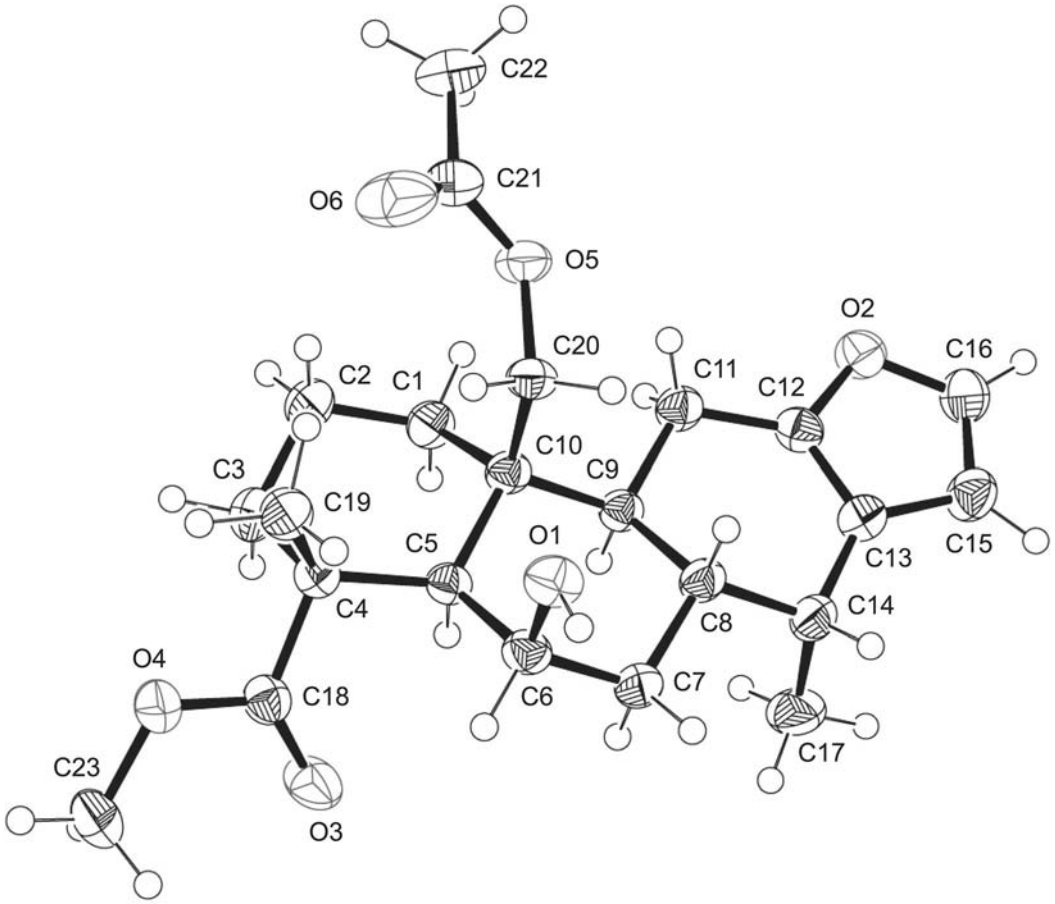

Fig. 1. *ORTEP Drawing of the molecular structure of* **1**

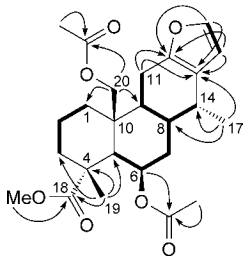

Fig. 2. *^{1}H,^{1}H-COSY* (—) *and key HMBC* (H → C) *correlations of* **2**

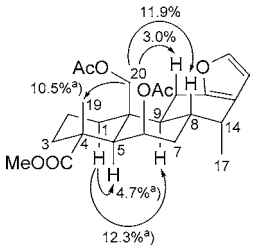

Fig. 3. *Key NOEs of* **2**. [a]) Observed in C_6D_6.

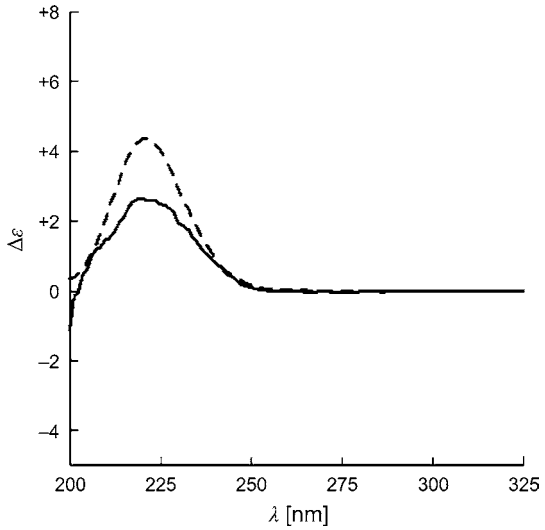

Fig. 4. *Comparison of experimental* (solid line) *and calculated* (dashed line) *ECD spectra of* **2** *in MeOH*

(**2**) was established as the 6-acetate of **1** and also the 14-deoxy analog of caesaljaponin A [4].

Supporting information associated with this article can be found online.

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Experimental Part

General. Thin layer chromatography (TLC): precoated *Kieselgel 60 F_{254}* plates (SiO_2; *Merck*, Darmstadt, Germany). Column chromatography (CC): SiO_2 (40 – 63 μm; *Merck*, Darmstadt, Germany). Optical rotations: *JASCO P-2200* polarimeter. UV Spectra: *JASCO V-630* spectrometer; λ_{max} (log ε) in nm. ECD Spectra: *JASCO J-725* spectropolarimeter; λ_{max} ($\Delta\varepsilon$) in nm. IR Spectra: *JASCO FT/IR-6300* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *JEOL A400* spectrometer (400 and 100 MHz, resp.); δ in ppm rel. to residual solvent peak (δ(H) 7.26, δ(C) 77.0), *J* in Hz. HR-ESI-FT-MS: *Thermo Fisher Scientific LTQ Orbitrap XL* mass spectrometer at the Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University; in *m/z*.

Plant Material. Seeds of *C. decapetala* var. *japonica*, which grows naturally on the river side in Hiroshima Prefecture, Japan, were collected in April 2014. A voucher specimen (No. 201404CJ) was deposited with the Laboratory of Natural Product Chemistry, Graduate School of Biosphere Science, Hiroshima University, Japan.

Extraction and Isolation. Cotyledons (48 g) separated from the seeds of *C. decapetala* var. *japonica* were cut into small pieces and extracted with MeOH. The MeOH extract was partitioned between hexane and MeOH. The MeOH-soluble portion was evaporated and suspended with H_2O. The suspension was extracted with AcOEt to yield an AcOEt-soluble portion. The AcOEt-soluble portion (1.1 g) was subjected to CC (SiO_2; AcOEt/hexane 0 : 100 to 100 : 0) to afford eleven fractions, *Frs. 1 – 11. Fr. 5* (15 mg) was purified by CC (SiO_2; AcOEt/hexane 0 : 100 to 15 : 85), affording **2** (10 mg, 0.02%). *Fr. 8* (21 mg) crystallized from hexane/CH_2Cl_2, affording **1** (9 mg, 0.02%).

Caesalpinista B (= *Methyl (4*R,*4a*R,*5*R,*6a*S,*7*R,*11a*S,*11b*S*)-11b-[(Acetyloxy)methyl]-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-5-hydroxy-4,7-dimethylphenanthro[3,2-*b*]furan-4-carboxylate*; **1**). Colorless crystals. M.p. 153.6 – 155.8. $[\alpha]_D^{20} = +24$ ($c = 0.12$, MeOH). UV (MeOH): 216 (3.65). ECD (MeOH): 219 (+4.99). IR (KBr): 3547, 1733, 1711, 1508, 1455. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-FT-MS: 427.2088 ($[M+Na]^+$, $C_{23}H_{32}NaO_6^+$; calc. 427.2091).

X-Ray Crystallographic Analysis of **1**[1]). Data collection was performed with a *Bruker SMART-APEX II ULTRA CCD* area detector with graphite monochromated CuK_α radiation (λ = 1.54178 Å). *Crystallographic Data:* $C_{23}H_{32}O_6$; $M_r = 404.50$; crystal size, $0.20 \times 0.20 \times 0.20$ mm; $T = 173$ K; trigonal; space group, $P3_1$; $Z = 3$; $a = 11.56235(9)$, $b = 11.56235(9)$, $c = 13.62422(11)$ Å; $\alpha = \beta = 90$, $\gamma = 120°$; $V = 1577.37(3)$ Å^3; $D_x = 1.277$ g cm^{-3}; $F(000) = 654$; 14237 reflections collected, 3819 independent reflections ($R_{int} = 0.0163$). The final R^1 value was 0.0321 ($I > 2\sigma(I)$), and the final wR^2 value was 0.0844 ($I > 2\sigma(I)$). The final R^1 value was 0.0322 (all data), and the final wR^2 value was 0.0845 (all data), the absolute structure parameter (*Flack* parameter) was 0.07(3).

Hydrolysis of **1**. K_2CO_3 Powder (60 mg) was added to a stirred soln. of **1** (3 mg) in MeOH (3 ml). After stirring the suspension at r.t. for 3 d, the mixture was purified by CC (SiO_2; AcOEt/hexane 1 : 4) to give **3** (2 mg).

Caesalpinista A (= *Methyl (4*R,*4a*R,*5*R,*6a*S,*7*R,*11a*S,*11b*S*)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-Dodecahydro-5-hydroxy-11b-(hydroxymethyl)-4,7-dimethylphenanthro[3,2-*b*]furan-4-carboxylate*; **3**). Colorless oil. $[\alpha]_D^{20} = +20$ ($c = 0.16$, MeOH). UV (MeOH): 218 (3.78). ECD (MeOH): 218 (+5.47). IR (KBr): 3420, 2923, 1721, 1647, 1464. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-FT-MS: 385.1987 ($[M+Na]^+$, $C_{21}H_{30}NaO_5^+$; calc. 385.1986).

Deoxycaesaljaponin A (= *Methyl (4*R,*4a*R,*5*R,*6a*S,*7*R,*11a*S,*11b*S*)-5-(Acetyloxy)-11b-[(acetyloxy)methyl]-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-4,7-dimethylphenanthro[3,2-*b*]furan-4-carboxylate*; **2**). Colorless oil. $[\alpha]_D^{25} = -6.7$ ($c = 0.12$, MeOH). UV (MeOH): 218 (3.50). ECD (MeOH): 218 (+2.57). IR (film): 1733, 1507, 1457. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-FT-MS: 469.2195 ($[M+Na]^+$, $C_{25}H_{34}NaO_7^+$; calc. 469.2197).

Hydrolysis of **2**. Hydrolysis of **2** (4 mg) was conducted in a manner similar to that of **1**, affording a hydrolysate (2 mg). The specific optical rotation, UV, ECD, IR, ^{1}H- and ^{13}C-NMR, and HR-ESI-FT-MS data were identical to those of **3**.

ECD Calculation of **2**. The theoretical ECD spectrum of **2** was obtained by a typical calculation procedure [14] described as follows: the conformational search of **2** with the expected absolute configuration was performed using CONFLEX 7 [15 – 17] with MMFF 94S (2010-12-04 HG) as force field on a commercially available PC (operating system, Windows 7 Professional SP1 64-bit; CPU, Quad-Core Xeon E3-1225 processor 3.10 GHz, RAM 8 GB), affording 208 stable conformers. Three outstandingly stable conformers – 47.3, 37.7, and 13.2% of abundance – were further optimized using GAUSSIAN 09 software [18] at the approximation level of B3LYP/6 – 31G(d) with the assumption of MeOH as the solvent with a polarizable continuum model (PCM) on a PC (operating system, CentOS a Linux; CPU, 2 Intel Xeon 3 5550 processors 2.67 GHz, RAM 24 GB). The population of the obtained conformers was analyzed by considering their *Boltzmann* distribution at 298 K based on their internal and vibrational energies. All obtained conformers were subjected to time-dependent simulations with the triple-zeta basis set TZVP with the hybrid functional CAM-B3LYP with the assumption that MeOH is the solvent with PCM. For each conformer, after assuring the reproduction of the experimental UV peak at 218 nm, the resultant rotational strengths were converted into *Gauss*ian curves (bandwidth $\sigma = 3000$ cm^{-1}) and correctively summed to give the ECD spectrum.

[1]) CCDC-1409080 contains the supplementary crystallographic data for this article. These data can be obtained free of charge from the *Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif.

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