Authenticity Assessment of γ- and δ-Decalactone from *Prunus* Fruits by Gas Chromatography Combustion/Pyrolysis Isotope Ratio Mass Spectrometry (GC-C/P-IRMS)

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Authenticity assessment of γ-decalactone (**1**) and δ-decalactone (**2**) from peach (*Prunus persica* var. *persica*), apricot (*Prunus armeniaca*), and nectarine (*Prunus persica* var. *nectarina*) was performed using gas chromatography-isotope ratio mass spectrometry (GC-IRMS) in the combustion (C) and pyrolysis (P) mode. In addition, commercially available synthetic (nature-identical) **1** and **2** as well as biotechnologically produced samples (declared to be "natural") were characterized by their δ^2H_{V-SMOW} and $\delta^{13}C_{V-PDB}$ values. For the *Prunus* fruits under study, rather narrow ranges of $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data of **1**, varying from −34.6‰ to −38.4‰ and −160‰ to −206‰, respectively, were obtained. Synthetic references of **1** showed $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data ranging from −27.4‰ to −28.3‰ and −151‰ to −184‰, respectively. Samples of **1** declared to be "natural" exhibited ranges from −28.1‰ to −29.2‰ and −192‰ to −286‰ for $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW}, respectively. For **2** from peach, apricot, and nectarine, $\delta^{13}C_{V-PDB}$ values ranging from −34.0‰ to −37.9‰ were determined; the δ^2H_{V-SMOW} values ranged from −171‰ to −228‰. The $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data for synthetic **2** were −28.2 ‰ and −171‰, respectively, that is, similar to those of **2** from "natural" origin, ranging from −27.7‰ to −30.1‰ and −185‰ to −230‰ for $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW}, respectively. GC-C/P-IRMS allowed clear-cut analytical differentiation of the synthetic and "ex-plant" origin of **1** and **2**, whereas narrow ranges of $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data were found for samples of synthetic and "natural" origin.

KEYWORDS: Authenticity; $^{13}C/^{12}C$ ratio; γ-decalactone; δ-decalactone; $^2H/^1H$ ratio; IRMS; isotope ratio mass spectrometry; lactones, *Prunus*

INTRODUCTION

Peach (*Prunus persica* var. *persica*), apricot (*Prunus armeniaca*), and nectarine (*Prunus persica* var. nectarina) are representative fruits of the Rosaceae family commercially involved in an important industrial market. The pleasant flavor of these fruits has early attracted the attention of flavor research (*1−4*). The characteristic aroma was attributed mainly to γ- and δ-lactones detected as major components, and, therefore, lactones have found particular attention in the food industries as flavoring agents.

To date, several organic routes have been developed to produce γ- and δ-lactones (*5*); however, an effective route to optically active lactones is still a target in organic synthesis (*6, 7*). As to biotechnological procedures, fatty acid derivatives are mostly used as substrates of the enantioselective γ-decalactone (**1**) and δ-decalactone (**2**) syntheses, and various microorganisms

such as *Sporobolomyces* sp., *Mucor* sp., baker's yeast, *Fusarium* sp., *Yarrowia lipolytica*, and others are propagated as suitable biocatalysts for the biogenerated production (*8−10*). Thus, biotechnologically produced **1** and **2** labeled as "natural" may have been produced from various sources using several microorganisms. In addition, the term "natural" also includes "ex plant" production.

With regard to flavor authenticity, both enantioselectivity and isotope discrimination during flavor genesis can serve as basic parameters provided that suitable methods and comprehensive data from authentic sources are available. The state-of-the art has been reviewed recently by Mosandl (*11*). Whereas enantiodifferentiation is applied extensively, isotope ratio mass spectrometry (IRMS), already used previously in the combustion (C) mode in combination with gas chromatography (GC-IRMS) for the determination of $^{13}C/^{12}C$ ratios of flavor compounds (*12−14*), has recently gained increased attention by the introduction of the pyrolysis (P) mode (*15−19*). The latter has been applied successfully in the authenticity assessment of flavor compounds by monitoring, in particular, of their $^2H/^1H$ ratios, for example, benzaldehyde (*20*), citral (*21*), (*E*)-2-hexenal, (*E*)-2-hexenol, and

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decanal (*22*), linalool and linalyl acetate (*22, 23*), as well as several aromatic volatiles (*24−26*). In this study, we undertook the authenticity assessment of **1** and **2** from peach, apricot, and nectarine fruits by means of $^{13}C/^{12}C$ and $^{2}H/^{1}H$ isotope ratios measured by GC-C/P-IRMS analysis.

MATERIALS AND METHODS

Materials. Fresh, ripe peaches (*Prunus persica* [L.] Batsch), nectarines (*Prunus persica* Batsch var. *nucipersica* [L.] C. K. Schneid), and apricots (*Prunus armeniaca* [L.]), harvested in Greece, Italy, France, Hungary, and Spain, were obtained from local markets in Würzburg, Germany, in the period between May and August, 2003. Samples of "natural" and synthetic (nature-identical) γ-decalactone **1** ($n = 7$) and δ-decalactone **2** ($n = 4$) were obtained from Hasegawa, Kanagawa, Japan; Symrise, Holzminden, Germany; Givaudan, Dortmund, Germany; as well as Sigma-Aldrich, Steinheim, Germany; Fluka, Deisenhofen, Germany; and Roth, Karlsruhe, Germany.

Chemicals of p.a. quality were purchased from Sigma-Aldrich, Steinheim, Germany. Solvents were redistilled before use.

Sample Preparation. "Natural" and synthetic **1** and **2** were dissolved (1 mg/mL) in diethyl ether before gas chromatographic analyses.

Fruits of various origin (peaches, $n = 7$; apricots, $n = 5$; nectarines, $n = 6$) were washed with distilled water, quartered, stoned, and blended for 20 s in a Waring blender. A 1200−1800 mL aliquot of the slurry was placed in a 4-L Pyrex round flask and then immediately subjected to simultaneous distillation extraction (SDE) for 2.5 h using 90 mL of a pentane−diethyl ether mixture (1:1, v/v).

Model experiments comprised SDE of solutions of **1** and **2** (each 50 mg in 800 mL of distilled water).

All of the extracts were dried over anhydrous sodium sulfate, filtered, and carefully concentrated to approximately 1 mL using a Vigreux column (45 °C).

Gas Chromatography−Mass Spectrometry (GC−MS). An Agilent 6890 Series gas chromatograph with split injection was directly coupled to a 5973 MSD mass spectrometer (Agilent Technologies Inc., CA). The GC was equipped with a 30 m × 0.25 mm i.d., 0.25 μm, DB-Wax (J&W, Agilent, Waldbronn, Germany) fused silica capillary column. Analytical conditions were as follows: 1 μL split injection (1:20); flow rate of carrier gas (helium), 2 mL/min; injector temperature, 250 °C; temperature program 50−200 °C at 5 °C/min. The temperature of the ion source was 220 °C, and that of the transfer line was 200 °C. The electron energy for the EI mass spectra was 70 eV, and the cathodic current was 4.1 mA. Identification was performed by comparison of linear retention indices and mass spectrometric data of sample constituents with those of authentic reference compounds.

Gas Chromatography-Combustion- and Gas Chromatography-Pyrolysis-Isotope Ratio Mass Spectrometry (GC-C/P-IRMS). A Finnigan Delta plus XL mass spectrometer was coupled by an open-split via a combustion/pyrolysis (C/P) interface to an HP 6890 gas chromatograph (GC). The GC was equipped with a 60 m × 0.32 mm i.d.; 0.25 μm, fused silica DB-Wax (J&W) capillary column. Aliquots of 1 μL were injected splitless at 250 °C. The temperature program was 50−200 °C at 5 °C/min, and helium flow of 2 mL/min was used.

Combustion to CO_2 was performed using an oxidation reactor (Al_2O_3; 0.5 mm inner diameter, 1.5 mm outer diameter, 320 mm) with Cu/Ni/Pt metal (240 mm × 0.125 mm) set at 960 °C. Water separation was performed using a Nafion membrane. The separated compounds eluting from the GC column yield H_2 in the pyrolysis interface. The effluent from the GC passes through a ceramic tube (Al_2O_3, 0.5 mm i.d., l = 320 mm) kept at 1440 °C.

In addition, coupling $^{13}C/^{12}C$ elemental analyzers (EA) (Euro Vector EA 3000, Milano, Italy); temperature 1000 °C; and $^{2}H/^{1}H$ (HT Sauerstoff Analysator HEKAtech, Wegberg, Germany) to the IRMS was realized for control determinations of reference substances.

Daily instrument performance checks were carried out by measuring reference samples with known $^{13}C/^{12}C$ and $^{2}H/^{1}H$ ratios. Stability check of the used reference gases was continuously performed by measuring International Atomic Energy Agency (IAEA, Vienna, Austria) standards with defined $^{13}C/^{12}C$ and $^{2}H/^{1}H$ ratios (for $^{13}C/^{12}C$ IAEA-CH-7 and for $^{2}H/^{1}H$ IAEA-CH-7, NBS 22 oil, and V-SMOW).

Table 1. $\delta^{2}H_{V-SMOW}$ and $\delta^{13}C_{V-PDB}$ Values [‰] of γ-Decalactone (**1**) and δ-Decalactone (**2**) from Peach, Apricot, and Nectarine[a]

	1		**2**	
	$\delta^{2}H_{V-SMOW}$	$\delta^{13}C_{V-PDB}$	$\delta^{2}H_{V-SMOW}$	$\delta^{13}C_{V-PDB}$
Peach				
Springcrest, Spain	−202 ± 3	−38.1 ± 0.3	n.d.	−35.3 ± 0.3
Melocot, Spain	n.d.	−36.5 ± 0.3	n.d.	−36.2 ± 0.2
Rich May, Italy	n.d.	−35.4 ± 0.2	−209 ± 3	−34.9 ± 0.3
Flavorcrest, Italy	−195 ± 1	−37.9 ± 0.3	−197 ± 5	−37.0 ± 0.3
Springbell, Italy	−189 ± 2	−35.8 ± 0.3	n.d.	−34.3 ± 0.3
Royal Glory, France	−174 ± 1	−34.6 ± 0.3	−209 ± 3	−34.8 ± 0.3
Summerrich, France	−206 ± 1	−38.0 ± 0.3	−221 ± 2	−37.9 ± 0.3
Apricot				
Mauricio, Spain	−174 ± 2	−34.6 ± 0.3	−171 ± 5	−33.5 ± 0.3
Unknown, Greece	−170 ± 3	−35.5 ± 0.3	−228 ± 1	−34.1 ± 0.3
Pannonia, Hungary	−161 ± 2	−36.2 ± 0.2	−178 ± 5	−34.0 ± 0.3
Jumbocot, France	−195 ± 5	−37.7 ± 0.3	−185 ± 6	−35.9 ± 0.3
Thyrinthe, France	−162 ± 5	−35.4 ± 0.3	n.d.	−36.5 ± 0.3
Nectarine				
Bigtop, Spain	−190 ± 1	−36.6 ± 0.3	−201 ± 3	−34.8 ± 0.2
Laura, Italy	−175 ± 5	−36.9 ± 0.3	−201 ± 3	−35.7 ± 0.3
Springred, Italy	−190 ± 1	−38.1 ± 0.3	−216 ± 3	−35.7 ± 0.1
Emerard, France	−166 ± 2	−37.7 ± 0.3	−209 ± 1	−36.0 ± 0.3
Flavorgold, France	−205 ± 1	−38.4 ± 0.3	−227 ± 2	−37.2 ± 0.3
Jade, France	−160 ± 4	−35.6 ± 0.3	n.d.	−34.3 ± 0.3

[a] n.d. = not determined (insufficient amount).

The isotope ratios $^{2}H/^{1}H$ are expressed in per mil (‰) deviation relative to the V-PDB and V-SMOW international standards. Results are expressed in δ values as follows (for $\delta^{2}H$, corresponding formula is valid):

$$\delta^{13}C_{V-PDB} \text{ [‰]} = \left(\frac{R_{sample} - R_{V-PDB}}{R_{V-PDB}}\right) * 1000$$

R is the isotope ratio $^{13}C/^{12}C$ (corresponding for $\delta^{2}H$: $^{2}H/^{1}H$).

For $^{13}C/^{12}C$ and $^{2}H/^{1}H$ measurements, calibration of the IRMS was done with certified CO_2 ($\delta^{13}C_{V-PDB} = -24.90 \pm 0.2$‰) and H_2 ($\delta^{2}H_{V-SMOW} = -270 \pm 10$‰) reference gases (Messer Griesheim, Frankfurt, Germany).

Five-fold determination was carried out, and standard deviations were calculated. They were ±0.2−0.3‰ and ±5‰ for $\delta^{13}C_{V-PDB}$ and $\delta^{2}H_{V-SMOW}$ determinations, respectively. Additional peak recognition was performed by reference compounds and GC−MS registered under identical separation conditions as samples.

As it is well-known that determinations in the P-IRMS mode depend on the structure and the quantity of samples to be analyzed (*22, 28*), evaluation of the linear dynamic range for **1** and **2** was required. The $\delta^{2}H_{V-SMOW}$ linear dynamic ranges of **1** and **2** were determined via GC-IRMS using different sample amounts. Linearity ranges were found to be 1−4 μg on column.

To determine the $\delta^{2}H_{V-SMOW}$ values of **1** and **2**, the system reliability had further to be proven by measuring commercial references via the equipped EA. Comparison of the data recorded by EA-P-IRMS revealed good agreement with that determined by GC-P-IRMS analysis (data not shown). Finally, the influence of SDE sample preparation on the $^{2}H/^{1}H$ ratio checked by model SDE separations was found to be within the range of standard deviation and, thus, negligible (data not shown).

RESULTS AND DISCUSSION

γ-Decalactone (**1**) and δ-decalactone (**2**) were found among the major volatile constituents of the *Prunus* fruits under study. Apart from a few exceptions, in which the amounts of **1** and **2** were not sufficient, the lactones could be easily analyzed by GC-C/P-IRMS. The $\delta^{13}C_{V-PDB}$ and $\delta^{2}H_{V-SMOW}$ values recorded for **1** and **2** in peach, apricot, and nectarine fruits are outlined in **Table 1**. In **Table 2**, the corresponding δ-values determined for synthetic and "natural' samples of **1** and **2** are summarized.

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Table 2. δ^2H_{V-SMOW} and $\delta^{13}C_{V-PDB}$ Values [‰] of γ-Decalactone (**1**) and δ-Decalactone (**2**) Samples Declared To Be Synthetic and "Natural"
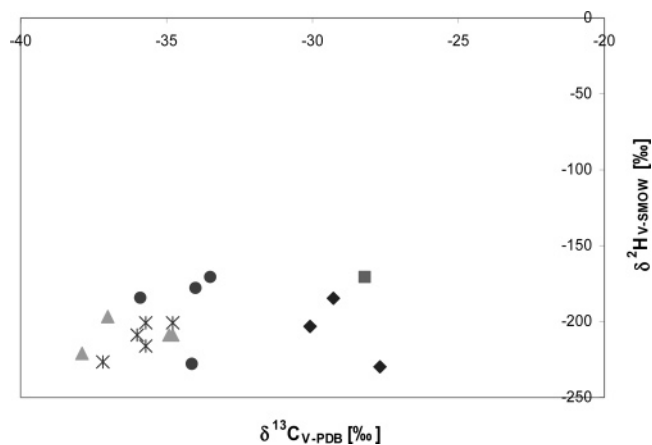
	δ^2H_{V-SMOW}	$\delta^{13}C_{V-PDB}$
1		
synthetic	−184 ± 2	−28.3 ± 0.1
synthetic	−151 ± 5	−27.4 ± 0.1
natural	−214 ± 2	−28.1 ± 0.1
natural	−230 ± 4	−29.2 ± 0.1
natural	−247 ± 2	−29.2 ± 0.1
natural	−192 ± 2	−28.3 ± 0.1
natural[a]	−286 ± 1	−29.0 ± 0.1
2		
synthetic	−171 ± 1	−28.2 ± 0.1
natural	−203 ± 1	−30.1 ± 0.1
natural	−230 ± 3	−27.7 ± 0.1
natural[a]	−185 ± 1	−29.3 ± 0.1

[a] Declared to be microbiologically produced.
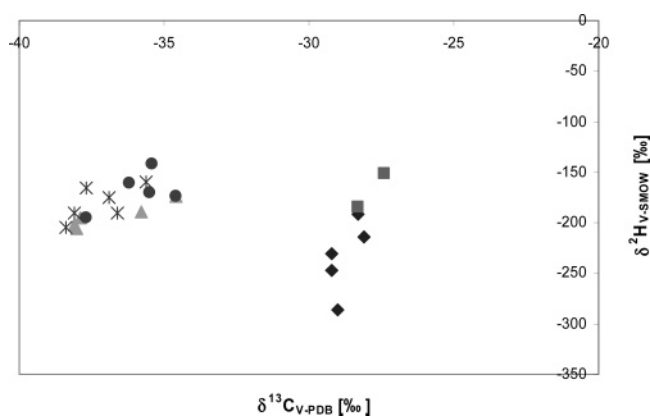
Figure 1. Correlation of $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} values [‰] of γ-decalactone from peach (▲), apricot (●), and nectarine (*) fruits, and synthetic (■) as well as natural (◆) reference. Standard deviations: ±0.2–0.3 and ±0.5‰ for $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} determinations, respectively.

γ-Decalactone (1). Studying **1** from peach, apricot, and nectarine, similar ranges of $\delta^{13}C_{V-PDB}$ values were observed by GC-C-IRMS analysis. For all of these *Prunus* fruits, the data of **1** ranged from −34.6‰ to −38.4‰, not allowing analytical distinction between the analyzed species. The recorded data are in good agreement with those published sporadically in the literature (*12*). An analogous situation was found for the δ^2H_{V-SMOW} values; a rather narrow range of −160‰ to −206‰ was obtained for the *Prunus* fruits under study. There is no literature information available about δ^2H_{V-SMOW} values of **1** from these fruits.

Synthetic references of **1** showed $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data ranging from −27.4‰ to −28.3‰ and −151‰ to −184‰, respectively. Samples of **1** declared to be "natural" exhibited ranges from −28.1‰ to −29.2‰ and −192‰ to −286‰ for $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW}, respectively. The measured $\delta^{13}C_{V-PDB}$ data correspond to that given in the literature; published data (*n* = 10) range from −24.4‰ to −30.2‰ for synthetic **1** (*12, 29−32*). For biotechnologically produced **1**, $\delta^{13}C_{V-PDB}$ data from literature range from −30.1‰ to −31.2‰ (*n* = 4) (*29, 30, 33*). There is no previous information available on δ^2H_{V-SMOW} values of **1**.

As can be seen from the correlation of $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data of **1** outlined in **Figure 1**, γ-decalactone originating from the peach, apricot, and nectarine fruits can easily be differentiated from that of synthetic and "natural" (biotechnological) origin. Decisive influence is given by the

Figure 2. Correlation of $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} values [‰] of δ-decalactone from peach (▲), apricot (●), and nectarine (*) fruits, and synthetic (■) as well as natural (◆) reference. Standard deviations: ±0.2–0.3 and ±0.5‰ for $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} determinations, respectively.

$\delta^{13}C_{V-PDB}$ values. Only small differences were found both for $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data of **1** from synthetic and "natural" origin. The database, however, is still too limited to draw final conclusions from this observation.

δ-Decalactone (2). As to the $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} values for **2**, from the *Prunus* fruits under study, a situation similar to that observed for **1** was obtained (**Table 1**). For **2** from peach, apricot, and nectarine, $\delta^{13}C_{V-PDB}$ values ranging from −33.5‰ to −37.9‰ were determined; analytical distinction between the species was not possible. Analogously, δ^2H_{V-SMOW} values ranged from −171‰ to −228‰ (in some fruit extracts $^2H/^1H$ values of **2** could not be determined because of its insufficient amount). An attempt to characterize isotopically several δ-decalactones of various origin was done more than 10 years ago by Fronza et al. using the natural abundance deuterium NMR technique to determine the site-specific $^2H/^1H$ isotopic ratios (*34*). The total deuterium content determined for the samples at that time appears to be very low as compared to the data reported for the samples described in the present work. Nevertheless, the differences displayed in the values of the site-specific $^2H/^1H$ ratios seem to provide a valid indication for distinguishing samples of synthetic and biotechnological origin, distinction which is not possible at the moment using the total $^2H/^1H$ and $^{13}C/^{12}C$.

The $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data for synthetic δ-decalactone (*n* = 1) were −28.2 ‰ and −171‰, respectively, that is, not clearly differentiated from those of **2** from "natural" origin (*n* = 3), ranging from −27.7‰ to −30.1‰ and −185‰ to −230‰ for $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW}, respectively.

As can be seen from the correlation of $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} data of **2** outlined in **Figure 2**, δ-decalactone originating from the fruits peach, apricot, and nectarine can be clearly differentiated from that of synthetic and "natural" (biotechnological) origin. Analogously to **1**, also for **2** decisive influence is given by the $\delta^{13}C_{V-PDB}$ values (**Figure 2**). In flavor authenticity assessment based on IRMS, such a strong influence of $\delta^{13}C_{V-PDB}$ values, at least in the group of volatiles from C3 plants, is rather scarce. As was recently shown by means of many examples, δ^2H_{V-SMOW} values are preferably used as a more reliable parameter for the authenticity assessment of plant volatiles because of the wideness in value and specificity of the values in each plant (*22, 28*).

Assessment of Mixtures of 1 (Ex Plant/Synthetic). Additional information for further authenticity evaluation was
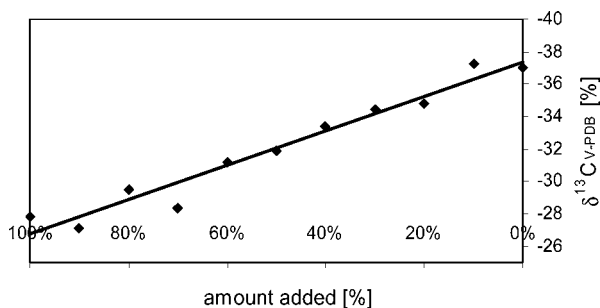
Figure 3. $\delta^{13}C_{V-PDB}$ values [‰] of δ-decalacton from nectarine (-37.0 ± 0.1‰) amounts of synthetic δ-decalacton (-27.4 ± 0.5‰ determined by elemental analyzer) subsequently added (0–100%).

achieved by adding synthetic **1** ($\delta^{13}C_{V-PDB} = -27.4$‰) stepwise (10%) to a peach aroma extract ($\delta^{13}C_{V-PDB}$ of **1** $= -37.0$‰) and subsequently measuring the resulting $\delta^{13}C_{V-PDB}$ value. The results obtained (**Figure 3**) show higher $\delta^{13}C_{V-PDB}$ values correlating with the amount of nature-identical **1** added.

Authenticity assessment of **1** and **2**, at present routinely performed by enantiodifferentiation, is limited in cases in which enantiomerically pure (or highly enriched) **1** or **2** is present. As demonstrated by the results of our study, GC-C-IRMS analysis will be the method of choice to differentiate analytically between the "ex *Prunus* plant" and biotechnological origin.

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