

A Vibrational Circular Dichroism Approach to the Determination of the Absolute Configurations of Flavorous 5-Substituted-2(5H)-furanones

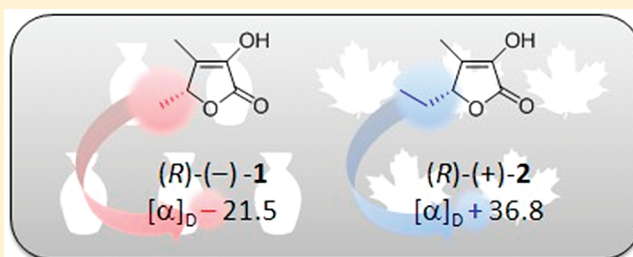
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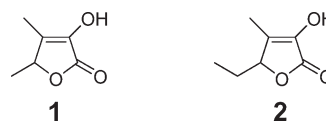
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S Supporting Information

ABSTRACT: Sotolon (**1**) and maple furanone (**2**) are naturally occurring chiral furanones. These 5-substituted-2(5H)-furanones are industrially significant aroma compounds due to their characteristic organoleptic properties and extraordinarily low odor thresholds. Each enantiomer of **1** and **2** was successfully obtained by preparative enantioselective supercritical fluid chromatography. The absolute configuration of **1** was confirmed as (*R*)-(-)-**1** and (*S*)-(+)-**1** by adopting the vibrational circular dichroism (VCD) approach. The absolute configuration of **2**, which has remained ambiguous since its discovery in 1957, was determined as (*R*)-(+)-**2** and (*S*)-(-)-**2** for the first time by the VCD technique. Surprisingly, the signs of the optical rotation of **2** are opposite of those of **1** regardless of their identical absolute configurations. This observation emphasizes the risk in absolute configurational assignments based on comparison of optical rotation signs of similar structures. Odor evaluation of the enantiomers of **2** revealed different odor intensities.



5-Substituted-2(*SH*)-furanones represented by 4,5-dimethyl-3-hydroxy-2(*SH*)-furanone (sotolon, **1**) and 5-ethyl-3-hydroxy-4-methyl-2(*SH*)-furanone (maple furanone, **2**) are industrially important aroma compounds that possess highly intense sugary sweet odors. Sotolon (**1**), originally found in aged Japanese sake in 1976 by Takahashi et al.,¹ was named after its typical odor of brown sugar, *sotou* in Japanese, and its lactone structure. Maple furanone (**2**), initially synthesized in 1957² and identified in hydrolyzed soy protein in 1980,³ has been named after its aroma, which is reminiscent of maple syrup. These volatile furanones are found in a variety of foodstuffs and drinks, such as cane sugar,⁴ maple syrup,⁵ rye,⁶ red raspberries,⁷ and wine,⁸ and can also be formed from certain amino acids.⁹ Because of their extremely low odor threshold values and characteristic odors, **1** and **2** play a significant role in flavor and have been widely used as raw materials in the flavor and fragrance industry for a long time. For instance, the odor threshold values are 1 and 0.01 parts per trillion (ppt) for **1** and **2**,⁹ respectively, the latter of which is one of the lowest odor threshold organic compounds known. Besides their flavor contribution, the odor of **1** was reported as an olfactory clinical index of maple syrup urine disease (MSUD),¹⁰ which is an autosomal recessive inherited disorder, for its typical odor from the body fluid of affected patients.



Naturally occurring aroma compounds often exhibit enantiomeric excesses, and the respective enantiomers possess different organoleptic perceptual characteristics and intensities.^{11,12} Sotolon (**1**) isolated from dried fenugreek seeds¹³ is optically active, and it is believed to be formed by the thermal degradation of an unusual amino acid, 4-hydroxy-*L*-isoleucine.¹⁴ On the other hand, maple furanone (**2**) is reported to be produced spontaneously from threonine,¹⁵ which implies natural occurrence of the chiral furanone.

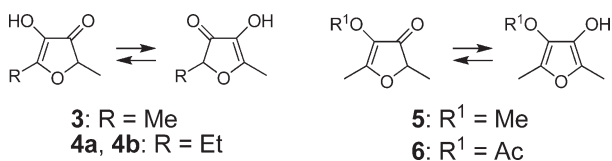
Despite their importance as flavor materials, there has been comparatively little work done to determine their absolute configurations. As for sotolon (**1**), the first asymmetric synthesis was accomplished by Mori et al. in 1983 starting from optically active tartaric acid as a chiral template.^{16a} Although it was followed by an alternative synthesis by Mosandl et al. in 1992,^{16b} there have been no further investigations confirming its absolute configuration. In the meantime, optically active

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maple furanone (**2**) was obtained in 1993 by using closed-loop recycling chromatography.¹⁷ However, neither asymmetric synthesis nor elucidations of the absolute configuration of **2** have been carried out so far. Unveiling their absolute configuration is essential for complete understanding of their biosynthetic pathways and the structural principle in the unique, intense maple syrup-like flavor.

Very recently, we have clarified the relationship between the absolute configurations and odor properties of the analogous flavorful 2-substituted-3(2*H*)-furanones such as 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF, **3**),^{18a} 2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2*H*)-furanone (EHMF, **4a** or **4b**),^{18b} 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (DMMF, **5**),^{18a} and 4-acetoxy-2,5-dimethyl-3(2*H*)-furanone (ADMF, **6**)^{18c} by utilizing the vibrational circular dichroism (VCD) technique. Although these compounds possess rather simple structures, the absolute configuration determination by conventional methods has been extremely difficult due to their unique keto–enol tautomeric structures and high chemical reactivity of the enol and carbonyl groups, which obstruct their derivatization toward X-ray crystallographic studies and the standard Mosher method. VCD measures the differential absorption of left versus right circularly polarized infrared radiation by molecular vibrational transition.¹⁹ Recently commercially available VCD equipment prompted the determination studies of the absolute configurations of chiral organic molecules^{20–23} as well as stereochemical analysis of biologically significant macromolecules, such as proteins²⁴ and carbohydrates.²⁵ In keeping with our chiroptical studies on the 2-substituted-3(2*H*)-furanones, the VCD method was applied to sotolon (**1**) and maple furanone (**2**), where the absolute configuration of the latter had not been defined. As a result, an interesting correlation between their structures and optical activities has been made. Moreover, odor evaluation of each enantiomer of **2** was carried out.



RESULTS AND DISCUSSION

CO₂ supercritical fluid chromatography (SFC)²⁶ is known to allow fine resolution even at a high flow rate due to the low viscosity and high diffusivity of CO₂. For preparative separation of the compounds, this method has been considered to be more efficient than HPLC because of its faster run time, inexpensive operating cost with CO₂, and less waste of the mobile phase liquid. In this case, it is noteworthy to mention that because CO₂ is vaporized spontaneously at the end of SFC, the minimum thermal workup operation avoids the risk of racemization and thermal decomposition from the removal of organic solvents as eluents. In our previous studies, we have already succeeded in the efficient chiral separation of similar furanones using the SFC technique.¹⁸ To accomplish high-throughput optical resolution of **1** and **2**, chiral CO₂ SFC was performed with chiral stationary phase columns. After several trials, optically active **1** and **2** were afforded by the use of CHIRALPAK IA columns using 2-propanol as an entrainer (e.g., **2**: α = 1.14) (Figure 1).

Semipreparative scale chromatography with multiple injections gave several tens of milligrams of each enantiomer. The

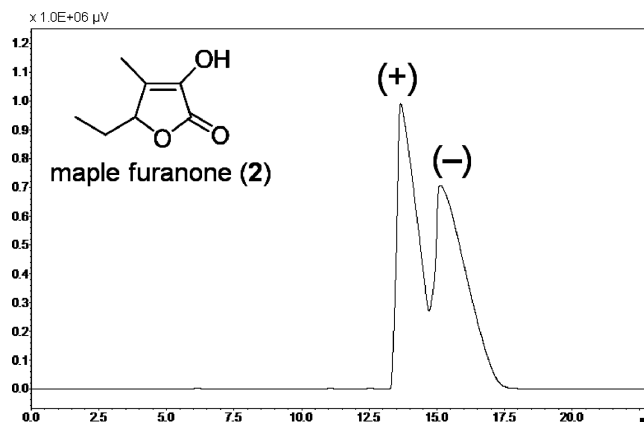


Figure 1. Optical resolution of **2** by enantioselective CO₂ SFC.

Table 1. Specific Rotation Values and Enantiomeric Excesses of Isolated Furanones

	$[\alpha]_D^{20}$	condition	ee (%)
(+)- 1	+13.4	c 0.30, CHCl ₃	88
(-)- 1	-21.5	c 0.30, CHCl ₃	96
(+)- 2	+36.8	c 0.30, CHCl ₃	99
(-)- 2	-35.5	c 0.30, CHCl ₃	98

obtained enantiomers exhibited specific rotation values shown in Table 1. The enantiomeric ratio was determined by chiral GC with a β-DEX325 column.²⁷ No racemization was observed during the workup, including the collection of fractions and the evaporation of the solvent.

The resulting four isomers were subjected to VCD studies. The IR and VCD spectra of each enantiomer were measured on a commercial Fourier transform VCD spectrometer in CCl₄ solution through a 72 μm path length cell with CaF₂ windows. Theoretical calculations of the VCD spectra of **1** and **2** were performed using density functional theory (DFT) at the B3PW91/6-31G(d,p) level. The initial geometries were generated with the CONFLEX search. The DFT conformational analysis revealed one and three low-lying conformers for **1** and **2**, respectively (Figure 2).

The observed IR and VCD spectra of sotolon (**1**) and the calculated IR and VCD spectra of (*R*)-**1** are shown in Figure 3. Observed VCD spectra of (+)-**1** and (-)-**1** are mirror images. The levorotatory enantiomer (-)-**1** showed an obvious positive Cotton effect around 1335 cm⁻¹, which can be attributed to a C–H bending at the stereogenic center. Interestingly, the corresponding VCD bands were observed in the previously measured VCD spectra of **4a**, **4b**, **5**, and **6**,¹⁸ which implied the possibility of a convenient stereoassignment of these types of furanones using the distinctive VCD signal as a reliable stereochemical marker.

The calculated IR spectrum of (*R*)-**1** showed good agreement with the observed IR spectra of both (+)-**1** and (-)-**1**, which suggests the high reliability of the calculation performed in this study. The calculated VCD signals of (*R*)-**1** agree well with those observed for (-)-**1** in their fingerprint regions (from 1500 to 1000 cm⁻¹). We therefore undoubtedly concluded the absolute configurations of **1** to be (*R*)-(-)-**1** and (*S*)-(+)-**1**, which is consistent with the results previously assigned by the synthetic works.¹⁶

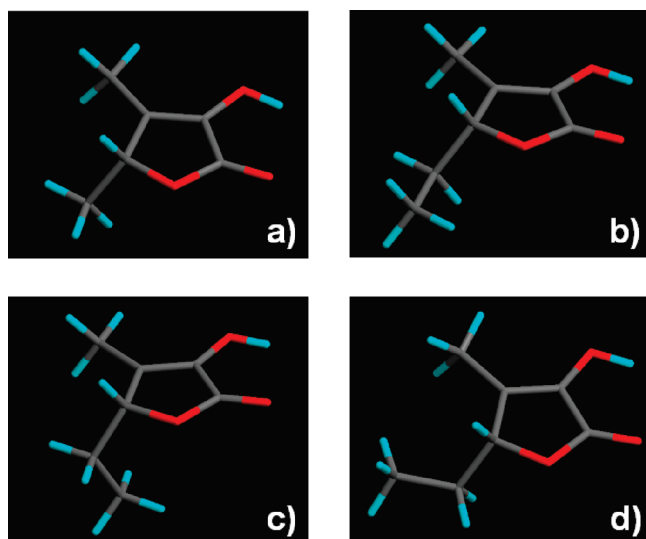


Figure 2. Most stable conformer of (*R*)-1 (a) and three stable conformers of (*R*)-2 (b, c, d).

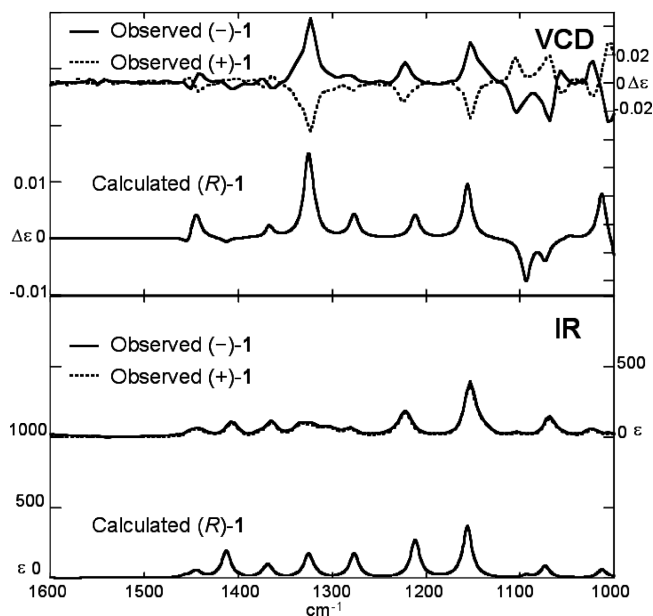


Figure 3. Comparison of IR (lower frame) and VCD (upper frame) observed spectra (CCl_4 , $c = 0.15 \text{ M}$, $l = 72 \mu\text{m}$) for ($-$)-1 with calculated spectrum for (*R*)-1.

Having confirmed the accuracy of the VCD technique for the absolute configuration determination of **1**, we then proceeded to the stereochemical study of maple furanone (**2**). Most of the observed characteristic IR absorptions of **2** are similar to those of **1** (Figure 4). On the contrary, the observed VCD spectrum of **2** is different from that of **1** especially around 1254 cm^{-1} , exquisitely reflecting their structural differences. Since the calculated VCD spectrum of (*R*)-**2** showed an excellent agreement with the observed VCD spectrum of (+)-**2**, configurations of **2** were successfully determined as (*R*)-(+)-**2** and (*S*)-(–)-**2** for the first time.

It should be noted that the signs of the specific rotation of **2** [(*R*)-(+)-**2** and (*S*)-(–)-**2**] are opposite those of **1** [(*R*)-(–)-**1** and (*S*)-(+)-**1**]. This might be a rare case in which slight

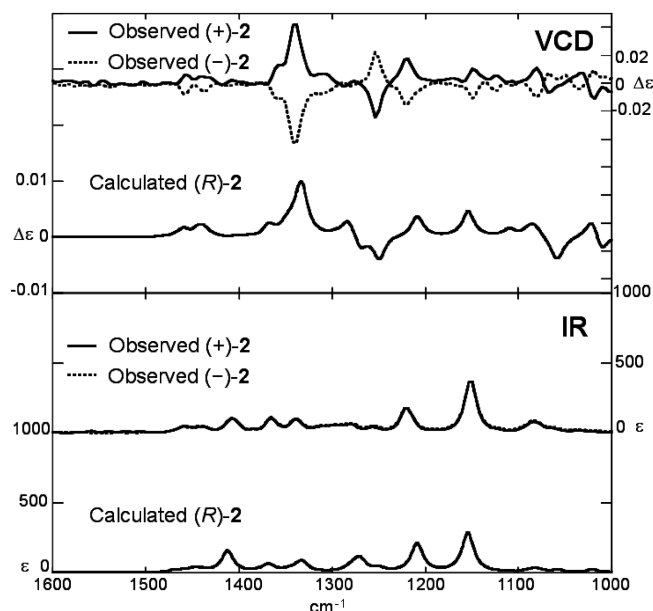


Figure 4. Comparison of IR (lower frame) and VCD (upper frame) observed spectra (CCl_4 , $c = 0.15 \text{ M}$, $l = 72 \mu\text{m}$) for (+)-**2** with calculated spectrum for (*R*)-**2**.

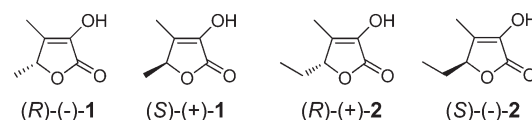


Figure 5. Absolute configurations of sotolon (**1**) and maple furanone (**2**).

differences of the substituent are responsible for a sign inversion of the $[\alpha]_D$ regardless of their identical absolute configurations. However, this inversion is also supported by our results of chiral GC analyses, in which (*R*)-(–)-**1** and (*R*)-(+)-**2**, namely, (*R*)-forms of both compounds, eluted earlier than their corresponding antipodes, reflecting their absolute configurations. Furthermore, Mosandl et al. also reported that (*R*)-(–)-**1** and (+)-**2** eluted earlier on their chiral GC analyses performed under similar conditions to our study, although the absolute configuration of **2** had not been determined.^{10,17} These experimental results ensure the stereochemical assignment in this study, and this unexpected and interesting finding illustrates the risk of the absolute configuration determination that relies only on the sign of optical rotation measured with a single wavelength.²⁸

Odor evaluation of each enantiomer of **2** was carried out. As expected, these enantiomers exhibited different odor characteristics. Our sensory evaluation study showed that (*R*)-(+)-**2** possessed a significantly intense maple syrup-like odor at 100 ppt solution in H_2O , while (*S*)-(–)-**2** was less intense. Our result is consistent with the results of previous work.¹⁰

In conclusion, we have accomplished a chiral separation of **1** and **2** by the SFC technique as well as the determination of the absolute configurations of (*R*)-(–)-**1**, (*S*)-(+)-**1**, (*R*)-(+)-**2**, and (*S*)-(–)-**2** through the use of the VCD technique. It must be emphasized that these furanones exhibit opposite optical rotation signs despite their same absolute configurations. This finding warns that even simple and rigid molecules with the same absolute configuration may have opposite optical rotation. We also investigated the structure–activity relationships between

absolute configurations and odor characteristics. We believe that the VCD method is also useful for chiroptical characterization of other aroma active compounds. In conjunction with our recent work on flavourous furanones,¹⁸ this study will facilitate further understanding of the basic molecular mechanism underlying our perception of their characteristic sweet odors.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a JASCO P-1020 polarimeter or a Perkin–Elmer 343 spectrometer. The chiral GC analyses were conducted on a GC2010 system (Shimadzu Corp.) equipped with a FID detector using a β -DEX325 column (30 m \times 0.25 mm i.d., 0.25 μ m film). Supercritical fluid chromatography was performed using a JASCO preparative SFC system equipped with a CHIRALPAK IA column (250 \times 20 mm i.d., Daicel Chemical Industries). Commercial grade racemic sotolon (**1**) was obtained from Takasago International Corp. (Tokyo, Japan). Racemic maple furanone (**2**) was purchased from Givaudan S. A. (Vernier, Switzerland). EtOAc, CHCl₃, and 2-propanol were purchased from Nacalai Tesque. CCl₄ was obtained from Wako Pure Chemical Industries, Ltd. Purity of each enantiomer was checked by the chiral GC system after optical resolution.

Optical Resolution of 4,5-Dimethyl-3-hydroxy-2(5H)-furanone (1**).** Racemic furanone **1** (0.15 g) in EtOAc (1.5 mL) was injected in a portion of 50 μ L into a preparative SFC system equipped with CHIRALPAK IA. Flow conditions were 25 mL/min CO₂ with 0.5 mL/min 2-propanol. The back pressure was kept at 15 MPa, and the UV signal was detected at 220 nm. The column oven temperature was kept at 25 °C. The optically active compounds were recovered with EtOAc as a line-wash solvent. After 10 injections were performed, (+)-**1** and (–)-**1** were isolated with 88% and 96% ee, respectively.

Optical Resolution of 5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone (2**).** Racemic furanone **2** (0.1 g) in EtOAc (1 mL) was injected in a portion of 150 μ L into a preparative SFC system equipped with CHIRALPAK IA. Flow conditions were 20 mL/min CO₂ with 0.5 mL/min 2-propanol. The back pressure was kept at 15 MPa, and the UV signal was detected at 220 nm. The column oven temperature was kept at 25 °C. The optically active compounds were recovered with EtOAc as a line-wash solvent. After five injections were performed, (+)-**2** and (–)-**2** were isolated with 99% and 98% ee, respectively.

Gas Chromatography. Enantiomeric ratio values of isolated optically active compounds were determined by a GC-2010 system (Shimadzu Corp. Kyoto) combined with a flame ionization detector (FID) equipped with a Supelco β -DEX 325 capillary column (30 m \times 0.25 mm i.d., 0.25 μ m film thickness, Sigma-Aldrich Corp., St. Louis, MO), at a constant 100 kPa pressure with He as a carrier gas. The initial oven temperature was set at 70 °C, which was then raised to 210 at 1 °C/min. Injector and detector temperatures were set to 250 °C.

Optical Rotation. Optical rotation was measured at 589 nm (sodium D-line) at 20 °C in a 10 mm cell. The values of optical rotation were determined by assuming a standard deviation of <3%.

VCD Spectroscopy. VCD spectra were measured on a Bomem/BioTools ChiralIR spectrometer with a resolution of 8 cm^{–1} at ambient temperature. All VCD spectra were recorded for 3 h. Samples were dissolved in CCl₄ and placed in a 72 μ m CaF₂ cell. The concentrations were as follows: (+)-**1**, 0.15 M; (–)-**1**, 0.15 M; (+)-**2**, 0.15 M; and (–)-**2**, 0.15 M. The IR and VCD spectra were corrected with a solvent spectrum obtained under the same experimental conditions and presented in molar absorptivity ϵ (L/mol cm).

Density Functional Calculation of (R)-1 and (R)-2. The CONFLEX searches^{29,30} based on molecular mechanics with MMFF94S force fields were performed for (R)-**1** and (R)-**2**. Since (R)-**1** has only one rotatable bond and (R)-**2** has just two rotatable

bonds, the number of effective conformers after the CONFLEX search were one for (R)-**1** and three for (R)-**2**. For these conformers, the geometry optimizations and harmonic frequency analysis were carried out using the DFT calculations at the B3PW91/6-31G(d,p) level. Using the frequency and intensity sets, the IR and VCD spectra were simulated for each conformer by convolution with the Lorentzian function. These spectra were averaged with the Boltzmann-weighted populations for (R)-**2**. The frequencies were scaled with a factor of 0.97. The Boltzmann-weighted populations were evaluated with the DFT energy corrected with the thermal free energy with respect to the vibrational motion. All DFT calculations were conducted using the Gaussian03 (revision C02)³¹ program code, and calculations of the IR and VCD spectra from the frequency and intensity sets were carried out with an in-house program code.

Sensory Evaluations. The sensory evaluation in this study was performed by expert panels of flavorists. The study was approved by the Institutional Review Board of Takasago International Corp.

ASSOCIATED CONTENT

S Supporting Information. Details of chiral GC analyses, NMR spectra of **2**, and VCD calculation of **1** and **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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DEDICATION

Dedicated to Dr. Koji Nakanishi of Columbia University for his pioneering work on bioactive natural products.

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