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# Computation-Assisted Structural Elucidation of Epoxyroussoeone and Epoxyroussoedione Isolated from *Roussoella japanensis* KT1651

Yuna Honmura,[†] Hiroto Takekawa,[†] Kazuaki Tanaka,[†] Hayato Maeda,[†] Tatsuo Nehira,*[,‡] Warren Hehre,[§] and Masaru Hashimoto*[,†]

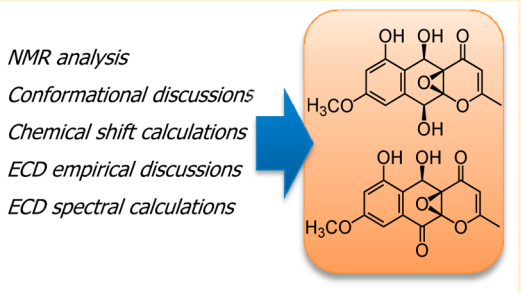
[†]Faculty of Agriculture and Bioscience, Hirosaki University, 3-Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan

[‡]Graduate School of Integrated Arts and Sciences, Hiroshima University, 1-7-1, Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8521, Japan

[§]Wavefunction, Inc., 18401 Von Karman Avenue, Suite 370, Irvine, California 92612, United States

Supporting Information

**ABSTRACT:** The structures of epoxyroussoenone (**1**) and epoxyroussoedione (**3**) isolated from a culture broth of *Roussoella japanensis* KT1651 were determined. Although NMR spectra provided insufficient structural information, computation of the theoretical chemical shifts with DFT EDF2/6-31G* enabled us to elucidate not only the planar structure, but also the relative configuration. Their ECD (electric circular dichroism) spectra suggested the absolute configurations, which were confirmed with time-dependent DFT calculations employing BHandHLYP/TZVP. The ECD calculations for other stereoisomers yielded obviously different spectral profiles, thus confirming the relative structures of **1** and **3**.




Remarkable progress in computer technology has enabled density functional theory (DFT) molecular orbital calculations of functionalized natural products via desktop computers.[1−5] Since molecular orbital calculations involving DFT methods deductively estimate the chemical and physical properties of molecules, these properties can be obtained without empirical information. Thus, theoretically derived molecular orbitals would be quite helpful in the structural determination for unique natural products, especially when the structure is difficult to determine if a standard spectroscopic technique such as NMR is used. From a practical viewpoint, however, in order to obtain the properties of interest with reasonable accuracy, we ought to choose appropriate methods that are feasible in terms of computation time and cost. We are now at the stage of refining the details such as the classes of approximations, basis sets, conformational search methods, and evaluation methods by data banking.

Although epoxyroussoeone (**1**) and epoxyroussoedione (**3**) possess only a few protons suitable for structural determination via their $^1$H NMR spectra, comparison of the experimental chemical shifts with the theoretical values of the candidate structures based on EDF2/6-31G*[6−8] allowed us to identify the most plausible structures for each. The ECD spectral results using BHandHLYP/TZVP[9] not only provided their absolute configuration, but also verified the relative configuration that we had assumed based on the chemical shift calculations. There were no inconsistencies between the NMR chemical shifts and ECD spectra.

## RESULTS AND DISCUSSION

During the exploration of useful biologically active fungal secondary metabolites with pharmaceutical applications, we focused on *Roussoella japanensis* KT1651[10] due to its antifungal activity against *Cochlibolus miyabeanus*. The fungus was isolated from a herbaceous bamboo, *Sasa veitchii*, at Kanagawa Prefecture, Japan, in 2004, and deposited at the National Institute of Agrobiological Sciences, Japan, as MAFF 239636 and at RIKEN, Japan Collection of Microorganisms, as JCM 13126. We recently disclosed tetracyclic diterpene fusicoccanes roussoellols A and B, from this fungus.[8] Further investigation led us to isolate epoxyroussoeone (**1**) and epoxyroussoedione (**3**) (Figure 1). Preliminary biological experiments indicated that **1** and **3** potently inhibited the growth of *C. miyabeanus* (MIC 1.0 μg/mL for both).

**Epoxyroussoeone (1).** Epoxyroussoeone (**1**) was obtained as a brown solid. Although the color was identified by TLC analysis as being due to polar impurities, the $^1$H NMR spectrum suggested that the **1** was mostly in a pure state (>95%

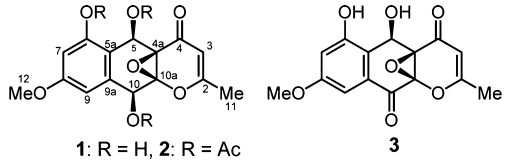



**Figure 1.** Structures of **1**−**3**.

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**Table 1. NMR Spectral Data of 1−3 in $CDCl_3$**

| | epoxyroussoenone (**1**) | | | acetate (**2**) | | epoxyroussoedion (**3**) | | |
|---|---|---|---|---|---|---|---|---|
| position | $\delta_C$, type | $\delta$ $^1$H ($J$ in Hz) | HMBC | $\delta_C$, type | $\delta$ $^1$H ($J$ in Hz) | $\delta_C$, type | $\delta$ $^1$H ($J$ in Hz) | HMBC |
| 2 | 170.26, C | | | 167.14, C | | 170.59, C | | |
| 3 | 104.53, CH | 5.62, s | 2, 4, 4a, 2-Me | 105.27, CH | 5.52, q (0.5) | 104.72, CH | 5.68, s | 2, 4, 4a, 5, 2-Me |
| 4 | 189.12, C | | | 185.16, C | | 187.92, C | | |
| 4a | 61.81, C | | | 63.09, C | | 60.56, C | | |
| 5 | 65.09, CH | 5.67, s | 5a, 6, 7, 9a, | 59.92, CH | 6.84, s | 65.09, CH | 5.72, s | 4a, 5a, 6, 7, 8, 9a |
| 5a | 108.51, C | | | 116.97, C | | 113.83, C | | |
| 6 | 158.39, C | | | 151.44, C | | 158.82, C | | |
| 7 | 103.31, CH | 6.49, d (2.5) | 5a, 6, 8, 9 | 110.33, CH | 6.61, d (2.6) | 110.72, CH | 6.78, d (2.6) | 5a, 6, 8, 9 |
| 8 | 161.30, C | | | 160.80, C | | 161.33, C | | |
| 9 | 104.70, CH | 6.79, d (2.5) | 5a, 7, 10 | 111.07, CH | 6.64, d (2.6) | 105.19, CH | 7.18, d (2.6) | 5a, 7, 8, 10 |
| 9a | 134.58 | | | 133.34, C | | 129.12, C | | |
| 10 | 68.45, CH | 5.17, d (9.9) | 9a, 5a | 68.15, CH | 6.70, s | 183.04, C | | |
| 10a | 88.25, C | | | 86.89, C | | 83.45, C | | |
| 11 | 21.05, $CH_3$ | 2.20, s | 2, 3 | 20.72, $CH_3$ | 2.06, d (0.5) | 21.26, $CH_3$ | 2.06, s | 2, 3, 10a |
| 12 | 55.41, $CH_3$ | 3.79, s | 8 | 55.82, $CH_3$ | 3.77, s | 55.88, $CH_3$ | 3.78, s | 8 |
| 5-OH | | 4.62, brs | | | | | 4.86, br | |
| 6-OH | | 8.52, brs | 5a, 6, 7 | | | | 8.65, br | |
| 10-OH | | 2.53, d (9.9) | | | | | | |
| 5−OCO | | | | 168.99, C | | | | |
| 6−OCO | | | | 169.76, C | | | | |
| 10−OCO | | | | 170.73, C | | | | |
| 5-OAc | | | | 21.13, $CH_3$ | 2.14, s | | | |
| 6-OAc | | | | 21.53, $CH_3$ | 2.29, s | | | |
| 10-OAc | | | | 21.12, $CH_3$ | 2.33, s | | | |

purity based on $^1$H NMR, see Supporting Information). Since **1** was labile under chromatographic conditions, further purification only resulted in sample loss. Recrystallization was also not helpful, as it yielded crystals with many dark spots. These operations probably accompanied the decomposition of **1** to produce the colored impurities. Thus, the yield of **1** was affected by the experimental operations (50−200 mg from 4.0 L of culture medium). We discovered that the sample was stable when stored in a freezer (−15 °C) for more than two months. However, once the sample was dissolved in a solvent ($CDCl_3$, acetone-$d_6$, or $CD_3OD$), a brown discoloration was observed. The HMQC and HMBC spectra were obtained with analyzable quality by completing the measurements within 12 h.

The NMR spectral data are summarized in Table 1. The $^{13}$C NMR spectrum of **1** gave 15 resonances. The LC−ESIMS afforded the ion at $m/z$ = 289.0718 ($C_{15}H_{13}O_6^+$), which was later assigned as the dehydrated ion ([MH − $H_2O$]$^+$, 289.0712). Acetylation under conventional conditions yielded triacetate (**2**), which successfully produced the protonated molecular ion at $m/z$ = 433.1124 ($C_{21}H_{21}O_{10}^+$, 433.1135). The ammonium adduct ion at $m/z$ = 450.1386 ([M + $NH_4$]$^+$, 450.1411), as well as the AcOH-eliminated ion at $m/z$ = 373.0912, ([MH − AcOH]$^+$, 373.0918), were also observed. These were helpful in confirming the above assignments. Triacetate **2** was sufficiently stable to allow for measurement of the NOEs.

Detailed NMR analysis of **1** disclosed several substructures: a 2-oxypropene unit (C1−C3); a carbonyl (C4); a benzene ring with a methoxy, a hydroxy, and two oxymethine groups (C5−C10); and two quaternary carbons (Figure 2). An *ortho*-relationship between H-7 and H-9 was established based on the coupling constant (2.5 Hz). The phenolic proton at C6 gave HMBC correlations with C5a, C6, and C7, which were helpful

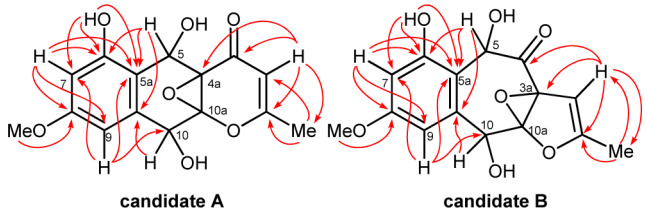


**Figure 2.** Candidate structures and HMBC correlations.

in assigning the aromatic ring. The $\delta$ values for the two quaternary carbons at 61.81 and 88.25 suggested an epoxide substructure with an additional oxygen attached to the latter carbon. This molecule should involve two rings, in addition to the benzene and epoxide moieties, when the index of hydrogen deficiency (IHD = 9) is considered. Formation of triacetate **2** revealed that **1** has three hydroxy groups. These findings suggest planar structures for benzochromene (candidate **A**) and benzocycloheptafuran (candidate **B**), as shown.

The IR absorption at 1655 cm$^{-1}$ (C═O stretching vibration) accorded with neither 2-cyclohexeneone (1685 cm$^{-1}$) nor 2-cycloheptanone (1716 cm$^{-1}$),[11] probably due to structural distortions, although candidate **A** showed a smaller deviation. All diastereomers of candidate **B** could be excluded by the theoretical chemical shifts calculations, as will be described later.

**Epoxyroussoedione (3).** *R. japanensis* also afforded epoxyroussoedione (**3**). Similar to the yield of **1**, that of **3** varied considerably by culture. The ESIMS of **3** yielded sodium and potassium adduct ions at $m/z$ = 327.0467 and 343.0212, respectively, suggesting the molecular formula $C_{15}H_{12}O_7$ ([M + Na]$^+$; 327.0481, [M + K]$^+$ 343.0220). As confirmation of this formula, the $^{13}$C NMR spectrum afforded 15 resonances. Therefore, **3** has two fewer hydrogens than **1**. Except for a few


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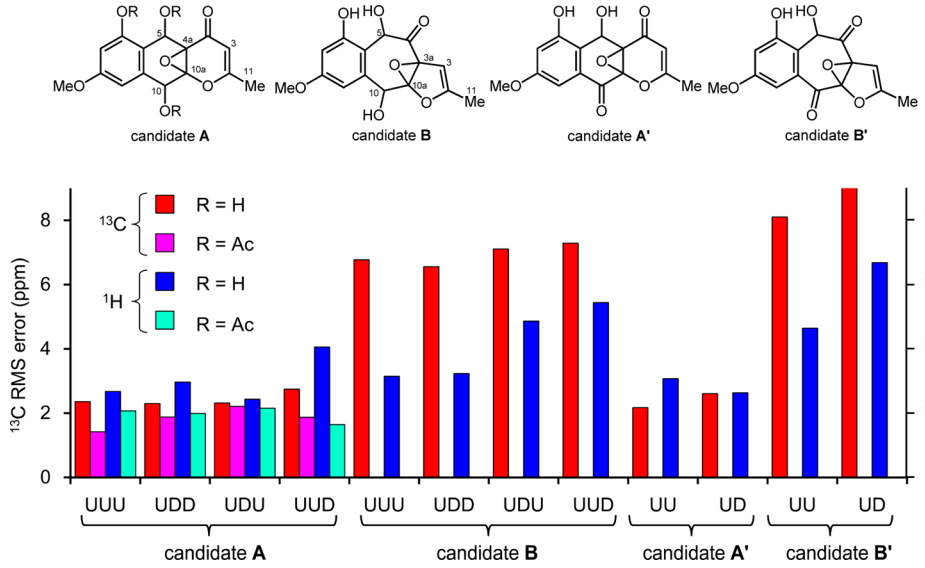


**Figure 3.** RMS errors (ppm) between experimental chemical shifts and those of candidate structures, based on DFT EDF2/6-31G*.

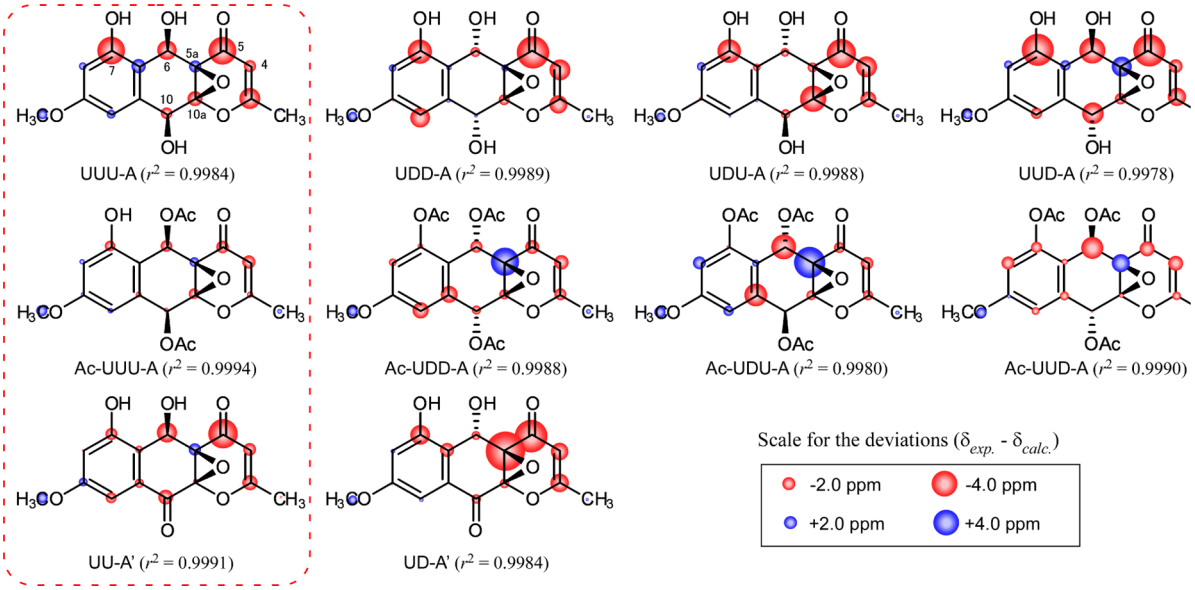


**Figure 4.** Localizations of $^{13}$C chemical shift deviations of calculated chemical shifts (EDF2/6-31G*) from experimental data (chemical shifts of acetyl groups are not plotted).

signals, the $^1$H and $^{13}$C NMR data of **3** resembled those of **1**. The C-10 resonance of **3** was observed at 183.4 ppm, whereas it was observed at 68.45 ppm in **1**. The H-10 signal was missing in the $^1$H NMR spectrum of **3**. When H-5 was excited, NOEs were observed both at the C5 alcoholic proton (4.86 ppm) and at the C6 phenolic proton (8.65 ppm) in the 1D NOESY spectra. This observation suggests that **3** is a C-10 keto-form of **1**. In **3**, the H-9 appeared at a higher frequency (ca. 0.4 ppm) than that in **1**, which also supports the C10 carbonyl functionality. However, at this stage, we cannot neglect the possibility that **3** is a C-10 keto-form of candidate **B**.

**Structural Results from Comparison of Theoretical Chemical Shifts.** Since we could not conclusively identify the planar structures of **1** and **3** with spectroscopic analysis, we next considered these using theoretical chemical shift calculations. All possible diastereomers of candidate **A** (UUU-**A**, UDD-**A**, UDU-**A**, UUD-**A**), candidate **B** (UUU-**B**, UDD-**B**, UDU-**B**, UUD-**B**), candidate **A**′ (UU-**A**′, UD-**A**′), and candidate **B**′ (UU-**B**′, UD-**B**′) were considered in these calculations. Acetates **2** (Ac-UUU-**A**, Ac-UDD-**A**, Ac-UDU-**A**, Ac-UUD-**A**) were also taken into consideration. The letters "U" and "D" refer to "up" and "down" for oxygen atoms at C4a, C5, and C10 (or for candidates **A**′ and **B**′ at C4a and C5), in the given order. Several calculations were then performed: (1) a conformational search, (2) fine structural optimization of the stable conformers with EDF2/6-31G*, (3) chemical shift calculations with the same approximation, and (4) correction of the calculated chemical shifts based on Boltzmann conformational distributions.[6−8] Notably, our preliminary calculations suggested that the conformational search with Merck Molecular Force Field (MMFF) resulted in low accuracy, and it missed several critical conformers in the case of candidate A. This limitation was probably due to structural distortion, because molecular mechanics are designed to reproduce unstrained structures. Therefore, conformational searches for all diastereomers of candidate **A** and their acetates were performed by employing


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J. Nat. Prod. XXXX, XXX, XXX−XXX

the Hartree−Fock (HF) method with the STO-3G basis set. The root-mean-square (RMS) errors (ppm) of the experimental data are summarized in Figure 3. Neither $^{1}$H nor $^{13}$C chemical shifts of the acetyl groups were involved in these analyses, because these signals are expected to appear in limited chemical shifts. Although the RMS errors of the $^{1}$H chemical shifts were less significant than those of the $^{13}$C chemical shifts, these results allowed us to successfully discard all stereoisomers of candidates **B** and **B**′. Regardless of the configuration, the $^{13}$C RMS values of candidates **A** and **A**′ were almost within the expected accuracy level of EDF2/6-31G* ($^{13}$C ca. 1.5 ppm, $^{1}$H 0.15 ppm).[12] Isomer UUD-A seemed to yield a slightly higher $^{13}$C RMS error.

We next determined the localization of chemical shift deviations for candidate **A**, its acetates, and the C10 keto-form candidate **A**′. The $^{13}$C deviations ($\Delta\delta$: $\delta_{exp.} - \delta_{calc.}$) are plotted on the structures in Figure 4. Those of the $^{1}$H signals were not considered because they involved only small numbers of protons. Acetyl-group carbons were also not considered.

Figure 4 shows that the $\Delta\delta$ of $^{13}$C varies significantly around the stereogenic centers. UUD-**A** shows a substantial $\Delta\delta$ over the whole molecule. This result is consistent with the findings based on the RMS errors. Although the chemical shift deviation of UUU-**A** is not outstandingly the smallest among the candidates for natural **1**, consideration of its acetates led us to identify Ac-UUU-**A** as the most plausible isomer. The case of candidate **A**′ is clearer: UU-**A**′ accords well with the experimental data, while UD-**A**′ shows a considerable chemical shift deviation at C4a. These findings allowed us to discard UD-**A**′. This rejection was reasonable given the fact that Ac-UUU-**A** (UUU-**A**) afforded the highest score among the isomers, because UU-**A**′ can be explained by the common biosynthetic pathway. However, the above discussion still contains some ambiguities.

We next studied the relative configuration of triacetate **2** with NOEs. As described, **2** was stable enough to perform NOE measurements. Triacetate **2** gave a characteristic NOE between H-5 and H-10, suggesting a *cis*-orientation for these protons. Although both models, Ac-UUU-**A** and Ac-UDD-**A**, may be consistent with this observation, the latter could be excluded due to its conformational property. As described in the discussion of the chemical shift computations, we obtained a series of stable conformers of the triacetate models. It was found that both the H-5 and H-10 of Ac-UUU-**A** featured pseudo-axial conformations in the stable isomers, while those of Ac-UDD-**A** featured a pseudo-equatorial conformation (Figure 5). Therefore, the NOE between H-5 and H-10 can be expected only in Ac-UUU-**A**. This expectation was confirmed by further NOE between acetyl groups on O-5 (2.12 ppm) and O-6 (2.28 ppm). When computations of Ac-UDD-**A** were performed by employing the initial conformations with pseudoaxial H-5 and H-10, structural optimization using EDF2/6-31G* only resulted in the flipping of the central cyclohexane ring to afford conformations with pseudo-equatorial H-5 and H-10. On the basis of the above findings, we concluded that UUU-**A** and UU-**A**′ are the relative structures of **1** and **3**, respectively.

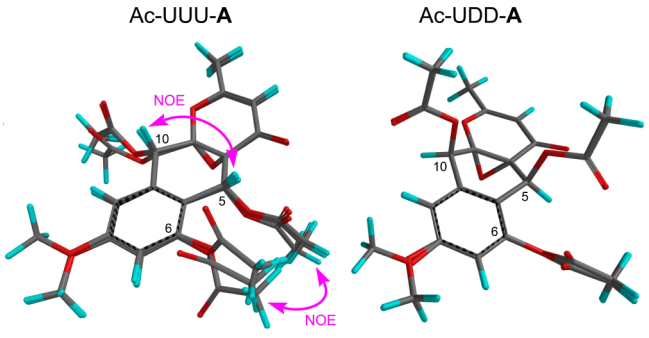


**Figure 5.** Stable conformers of **Ac-UUU-A** and **Ac-UDD-A** occupying more than 85% abundance, based on EDF2/6-31G*.

**Absolute Stereochemical Results from ECD Spectra.** The absolute configurations of **1** and **3** were studied using their ECD spectra, which verified the relative configurations obtained by the above investigation.

Both **1** and **3** generated distinct, positive Cotton effects at around 330 nm [**1**, $\Delta\varepsilon$ +1.6 (318 nm); **3**, $\Delta\varepsilon$ +4.9 (321 nm)], as shown in Figure 6. These can be assigned as the R-band (n → $\pi^*$ interaction) of the $\alpha,\beta$-unsaturated epoxyketone system.[13] Application of Snatzke's inverse-octant rule[14−19] suggested a (4a*S*)-configuration for **1**. Epoxyroussoedione (**3**) involves two $\alpha,\beta$-unsaturated epoxyketone systems with a common 4a,10a-epoxide. As shown in Figure 7, the epoxide group is located at the same behind-right-octant sector, based

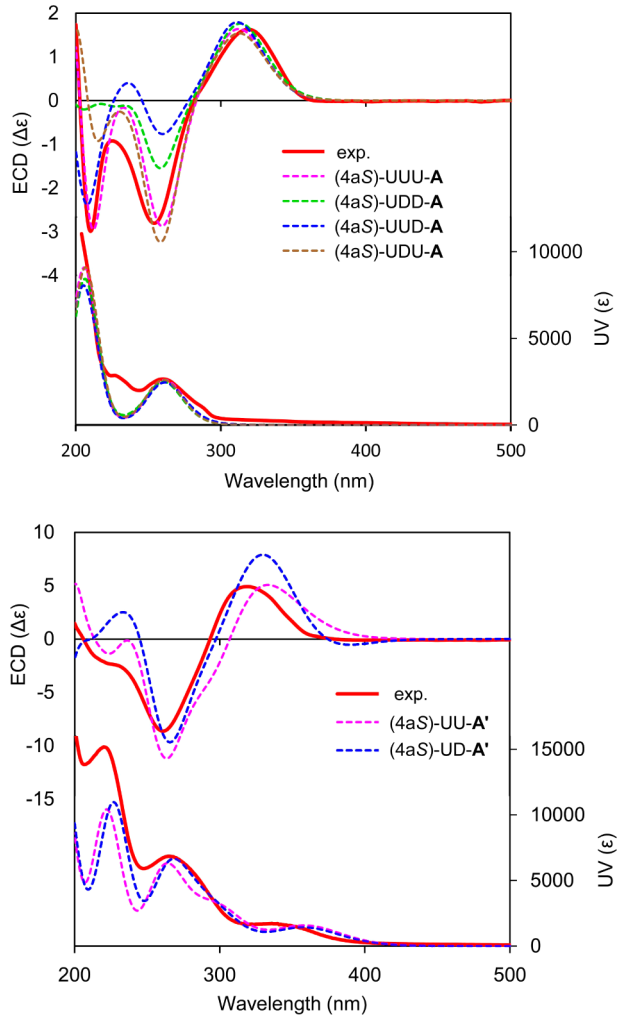


**Figure 6.** ECD spectra of experimental **1**, **3** ($CH_3CN$) and their calculated (4a*S*)-isomers estimated by TDDFT with BHandHLYP/TZVP. Solvent acetonitrile was considered in the calculations.



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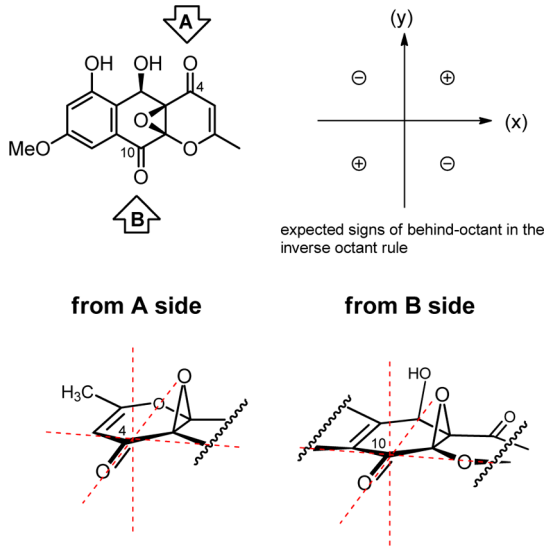


**Figure 7.** Configurational assignment of R-band in ECD spectrum.

on both C4 (from the A side) and C10 carbonyls (from the B side). Thus, both can contribute to a positive Cotton effect at the R-band.

Cotton effects at the K-bands ($\pi \rightarrow \pi^*$ interaction) were considered based on Sekiguchi's empirical rule. This rule dictates that the epoxysemiquinol system gives the K-band at around 245 nm and that the sign is attributable to the configuration of the hydroxy group.[15,18,20] Epoxyroussoedione (**3**) includes the epoxysemiquinol system in the central ring, which generates a negative Cotton effect at 261 nm. Application of Sekiguchi's rule indicated (5*R*)-configuration for **3**. Epoxyroussoenone (**1**) should also have a (5*R*)-configuration, based on structural and biosynthetic commonalities. These ECD analyses independently suggested (4a*S*)- and (5*R*)-configurations. The (4a*S*,5*R*)-forms for **1** and **3** are consistent with the relative configurations, UUU-**A** and UU-**A**′, that we determined above.

We confirmed these results with theoretical ECD calculations. A series of stable conformers used for NMR analysis was directly subjected to the ECD calculations employing BHandHLYP/TZVP.[9] Solvent acetonitrile was considered in these calculations. The considered conformers covered more than 90% of the Boltzmann distribution. After spectral correction based on the Boltzmann distribution, the wavelengths and ECD intensities were corrected based on the UV spectrum. The obtained calculated spectra were overlaid onto the experimental spectra (Figure 6).

Regardless of the C5 and C10 stereocenters, theoretical ECD spectra of (4a*S*)-isomers showed a positive Cotton effect at the R-band (around 320 nm). These showed close accordance with the empirical assignment based on the inverse-octant rule. The theoretical ECD spectra at lower wavelengths involving the K-band were dependent on the configurations at C5 and C10. Among the isomers, the spectra for (4a*S*)-UUU-**A** and (4a*S*)-UU-**A**′ accorded most strongly with those of natural **1** and **3**. Other isomers showed considerable discordance. These results not only determined their absolute configurations, but also verified their relative configurations. There were no contradictions across all stereochemical findings. The present results demonstrate that theoretical ECD calculations can also be applied to relative configurational investigations.

## CONCLUSION

As described, we determined the structures of epoxyroussoenone (**1**) and epoxyroussoedione (**3**), isolated from *R. japanensis* KT1651, hyphal swelling-inducing substances that act against *C. miyabeanus*. The present study demonstrates that DFT computations can play an indispensable and complementary role to NMR and ECD analyses during structure determination.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** The $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR spectra were recorded on a JEOL JNM-ECX500 spectrometer. Tetramethylsilane (0 ppm) was used as the internal standard when $CDCl_3$ was used as the solvent. Residual $^1$H and $^{13}$C in $CD_3OD$ ($^1$H 3.31 ppm, $^{13}$C 49.15 ppm) and $CD_3COCD_3$ ($^1$H 2.05 ppm, $^{13}$C 29.92 ppm) were employed as the internal standards when these were used as the solvents. Electrospray ionization (ESI) MS spectra were obtained from a HITACHI NanoFrontier LD spectrometer. Calibration was performed with tetrabutylammonium bromide (*m*/*z* 242.2848) and reserpine (*m*/*z* 609.2807).

**Isolation of the Fungus.** *Roussoella hysterioides* KT1651 was isolated from *S. veitchii* at Kanagawa Prefecture Japan in 2004. The fungal isolate was deposited at the National Institute of Agrobiological Sciences, Japan as MAFF 239636 and RIKEN Japan Collection of Microorganisms as JCM 13126.

*R. japanensis* KT1651 was cultured in a potato-sucrose medium (200 mL in a 500 mL baffled Erlenmeyer flask ×5) on a rotary shaker (110 rpm) at 25.8 °C for 14 d. After the medium was filtered under suction, the filtrate was extracted with EtOAc (500 mL × 2), and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to yield the crude extracts (685 mg). Silica gel column chromatography with EtOAc/benzene (50:50) produced the fraction containing epoxyroussoenone (**1**) and epoxyroussoedione (**3**) (190 mg) as a brown colored solid. The crude mixture of **1** and **3** was further subjected to silica gel column chromatography (20−40% AcOEt/benzene), to yield **1** (11.5 mg) and **3** (9.5 mg) as almost pure forms. Although TLC analysis indicated that both **1** and **3** included polar pigments as impurities, they were sufficiently pure to analyze the structures using NMR spectra (see Supporting Information). Further silica gel column chromatography was not effective due to their decomposition within the column. Recrystallization for X-ray analysis also failed, despite several attempts. The yields of **1** and **3** altered considerably due to their decomposition during purification. The $^1$H and $^{13}$C NMR data for these compounds in $CDCl_3$ are summarized in Table 1.

**Epoxyroussoeone (1:** $[\alpha]^{25}_D$ +62 (*c* 0.32, MeOH). ECD (1.7 × $10^{-4}$ mol/L, $CH_3CN$) $\Delta\varepsilon$ +1.6 (323 nm), −2.8 (253 nm), −0.8 (227 nm) −3.0 (211 nm), IR (film) 3280, 2925, 2850, 1655, 1650, 1625, 1435, 1400, 1325, 1145, 1000 cm$^{-1}$; $^1$H NMR ($CD_3COCD_3$) $\delta$ 2.13 (3H, s, $H_3$-11), 3.77 (3H, s), 5.24 (1H, s), 5.59 (1H, s), 5.70 (1H, s), 6.36, 6.78 (each 1H, d, *J* = 2.7 Hz), ($CD_3OD$) $\delta$ 2.14 (3H, s), 3.76 (3H, s), 5.14 (1H, s), 5.58 (1H, s), 5.59 (1H, d, *J* = 0.4 Hz), 6.37 (1H, d, *J* = 2.5 Hz), 6.69 (1H, dd, *J* = 0.4, 2.5 Hz); $^{13}$C NMR ($CD_3COCD_3$)$\delta$ 20.65, 55.57, 63.65, 65.10, 68.46, 89.61, ($CD_3OD$) $\delta$ 102.75, 104.80, 104.91, 111.57, 137.46, 159.20, 161.59, 169.82, $\delta$ 20.68, 55.80, 62.85, 66.06, 69.11, 90.45, 103.07, 104.89, 105.56, 113.96, 137.31, 159.21, 162.23, 170.23, 190.37; ESIMS *m*/*z* 289.0718, $[MH - H_2O]^+$; calcd 289.0712.

**Epoxyroussoeone (3):** $[\alpha]^{25}_D$ +58 (*c* 0.23, MeOH), ECD (6.6 × $10^{-4}$ mol/L, $CH_3CN$) $\Delta\varepsilon$ +4.9 (321 nm), −8.5 (263 nm), −2.2 (shoulder, 221 nm); IR (film) 3330, 2925, 2850, 1710, 1670, 1620, 1345 cm$^{-1}$; ESIMS *m*/*z* 327.0468, $[M + Na]^+$; calcd 327.0481, *m*/*z* 287.0546, $[MH - H_2O]^+$; calcd 287.0556.

**Acetylation of 1 to Triacetate 2.** Epoxyroussoenone (**1**, 12.0 mg) was stirred with acetic anhydride (50 mg) in pyridine (1.0 mL) at room temperature for 3 h. After concentration under reduced pressure, the residue was purified by preparative silica gel TLC (AcOEt/benzene 20:80) to yield **2** (9.8 mg) as an oil. $^1$H NMR ($CDCl_3$) $\delta$ 2.06 (3H, d,


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J. Nat. Prod. XXXX, XXX, XXX−XXX

$J$ = 0.5 Hz, $H_3$-11), 2.12, (3H, s, $H_3$−COOC5), 2.28 (3H, s, $H_3$−COOC6), 2.33 (3H, s, $H_3$−COOC10), 3.75 (3H, s, $H_3$-12), 5.50 (1H. q, $J$ = 0.5 Hz, H-3), 6.59 (1H, d, $J$ = 2.5 Hz, H-7), 6.62 (1H, d, $J$ = 2.5 Hz, H-9), 6.68 (1H, s, H-10), 6.84 (1H, s, H-5); $^{13}$C NMR (CDCl3) $\delta$ 20.50 (C-11), 20.91 ($\underline{C}H_3$COOC5), 21.30 ($\underline{C}H_3$COOC6), 21.30 ($\underline{C}H_3$COOC6), 20.89 ($\underline{C}H_3$COOC10), 55.60 (C-12), 59.70 (C-5), 62.87 (C-4a), 67.92 (C-10), 86.67 (C-10a), 105.04 (C-7), 110.10 (C-3), 110.85 (C-9), 116.75 (C-5a), 133.11 (C-9a), 151.22 (C-6), 160.57 (C-8), 166.92 (C-2), 168.77 ($CH_3\underline{C}OOC5$), 169.53 ($CH_3\underline{C}OOC10$), 170.50 ($CH_3\underline{C}OOC6$) 188.94 (C-5); HMBC H-3 → C-2, C-4a, C-11, H-5 → C-5a, C-6, C-9a, C-10, $\underline{C}OOC5$, H-7 → C-5a, C-6, C-8, C-9, H-9 → C-5a, C-10, C-7, C-8, H-10 → C-5a, C-9, C-9a, C-10a, $\underline{C}OOC10$, $H_3$-11 → C-2, C-3, $H_3$-12 → C-8, $H_3$−COOC5 → $\underline{C}OOC5$, $H_3$−COOC6 → $\underline{C}OOC6$, $H_3$−COOC10 → $\underline{C}OOC10$.

**Calculations.** Conformational searches, structural optimizations, and chemical shift calculations were conducted with Spartan 10 (64 bit) version 1.1.0 (Wave function, Irvine, CA), by using a customized PC (operating System, Windows7, Professional (64 bit); CPU, AMD Phenom(tm) II 970 processor 3.50 GHz, RAM 16 GB). The C5 and C10 (candidate **A**), or C4 and C5 (candidate **B**) were set to be puckered up and down in addition to the default atoms and bondings in the conformation search settings. The conformational searches for candidate **A** and its acetates were performed with STO-3G, while for candidate B, it was conducted with MMFF. Suggested stable conformers were further optimized with density functional theory (DFT), employing the EDF2 functional and 6-31G* basis set. Overlapping conformers were manually removed to generate sets of tentative stable conformers. Stable conformers within 3.0 kcal/mol of the global minimum conformation were subjected to chemical shift calculations with the same EDF2/6-31G*. The calculations were performed by assuming vacuum conditions without solvent effects. The theoretical chemical shifts were obtained after correction via the Boltzmann distribution. The statistical analyses were performed with Microsoft Excel 2010.

The ECD calculations were performed with Gaussian 09 (Revision A.02 by Gaussian, Wallingford, CT) using a PC (Operating System, CentOS Linux; CPU, 2 Intel Xeon 3 5550 processors 2.67 GHz, RAM 24 GB). The sets of stable conformations covering 90% abundances were subjected to time dependent (TD)DFT calculations with BHandHLYP/TZVP. Solvent acetonitrile was considered in these calculations by employing a polarizable continuum model (PCM). The resultant rotational strengths were converted into Gaussian curves (bandwidth sigma = 3500 cm$^{-1}$) after wavelength and intensity corrections with the experimental UV absorption peaks at 280 and 360 nm for compounds **1** and **3**, respectively. The final theoretical ECD spectra were obtained after correction via the Boltzmann distribution.

**Inhibition Assay against *C. miyabeanus*.** A series of suspensions of spores of *C. miyabeanus* and 0.1% sucrose containing 0.01, 0.1, 10, 100, and 1000 $\mu$g/mL of **1** and **3** were prepared in Petri dishes and incubated at 25 °C for 24 h. These were then observed under the microscope.

## ■ ASSOCIATED CONTENT

### Supporting Information

NMR spectra of **1**−**3**, summary of characteristic HMBC and NOE correlations in 3 molecules considered for chemical shift calculations, summary of the calculations (chemical shifts), summary of the calculations (coefficient determinations and standard errors). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/np500924n.

## ■ AUTHOR INFORMATION

### Corresponding Authors

*E-mail: hmasaru@cc.hirosaki-u.ac.jp. Tel./fax: +81-172-39-3782.

*E-mail: tnehira@hiroshima-u.ac.jp. Tel./fax: +81-82-424-6536/0759.

### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

(1) Tantillo, D. J. *Nat. Prod. Rep.* **2013**, *30*, 1079−1086.
(2) Sarotti, A. M. *Org. Biomol. Chem.* **2013**, *11*, 4847−4859.
(3) Smith, S. G.; Goodman, J. M. *J. Am. Chem. Soc.* **2010**, *132*, 12946−12959.
(4) Micco, S. D.; Chini, M. G.; Riccio, R.; Bifulco, G. *Eur. J. Org. Chem.* **2010**, *2010*, 1411−1434.
(5) Bringmann, G.; Bruhn, T.; Maksimenka, K.; Hemberger, Y. *Eur. J. Org. Chem.* **2009**, 2717−2727.
(6) Lin, C. Y.; George, M. W.; Gill, P. M. W. *Aust. J. Chem.* **2004**, *57*, 365−370.
(7) Yasumura, R.; Ashtekar, K. D.; Tonouchi, A.; Nehira, T.; Borhan, B.; Hashimoto, M. *Tetrahedron* **2013**, *69*, 9469−9474.
(8) Takekawa, H.; Tanaka, K.; Fukushi, E.; Nehira, T.; Hashimoto, M. *J. Nat. Prod.* **2013**, *76*, 1047−1051.
(9) Wahba, A. E.; Fromentin, Y.; Zou, Y.; Hamann, M. T. *Tetrahedron Lett.* **2012**, *53*, 6329−6331.
(10) The fungal name *Roussoella japanensis* used to be *Roussoella hysterioides* when we published roussoellols A and B. Recently it has been revised. Liu, J.-K.; Phookamsak, R.; Dai, D.-Q.; Tanaka, K.; Jones, E. B. G.; Xu, J.-C.; Chukeatirote, E.; Hyde, K. *Phytotaxa* **2014**, *181* (no. 1), 1−33.
(11) Spectral Database for Organic Compounds (SDBS) by National Institute of Advanced Industrial Science and Technology (AIST), Japan. (http://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi).
(12) Spartan '10 User Guide. http://downloads.wavefun.com/Spartan10Manual.pdf.
(13) Kwit, M.; Gawronski, J.; Boyd, D. R.; Sharma, N. D.; Kaik, M. *Org. Biomol. Chem.* **2010**, *8*, 5635−5645.
(14) Snatzke, G. *Tetrahedron* **1965**, *21*, 413−419.
(15) Sekiguchi, J.; Gaucher, M. *Biochem. J.* **1979**, *182*, 445−453.
(16) Fujimoto, H.; Nakamura, E.; Kim, Y.-P.; Okuyama, E.; Ishibashi, M.; Sassa, T. *J. Nat. Prod.* **2001**, *64*, 1234−1237.
(17) Sassa, T.; Yoshikoshi, H. *Agric. Biol. Chem.* **1983**, *47*, 187−189.
(18) Hirose, A.; Maeda, H.; Tonouchi, A.; Nehira, T.; Hashimoto, M. *Tetrahedron* **2014**, *70*, 1458−1463.
(19) Mohamed, I. E.; Gross, H.; Pontius, A.; Kehraus, S.; Krick, A.; Kelter, G.; Maier, A.; Fiebig, H.-H.; Konig, G. M. *Org. Lett.* **2009**, *11*, 5014−5017.
(20) Sekiguchi, J.; Gaucher, G. M. *Biochemistry* **1978**, *17*, 1785−1791.


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