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Absolute Configuration and ^{1}H NMR Characterization of Rosmaridiphenol Diacetate

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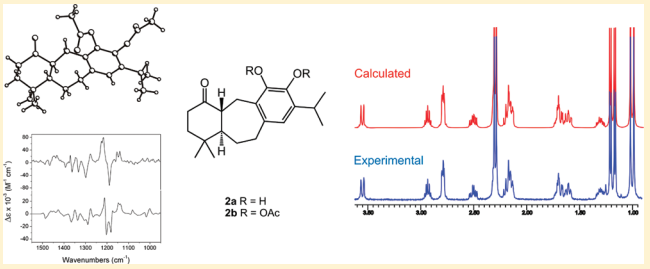
Supporting Information

ABSTRACT: The correction of patented structure **1** of rosmaridiphenol, an antioxidant isolated from rosemary, *Rosmarinus officinalis*, was made recently. The correct structure is proposed as 11,12-dihydroxy-8,11,13-icetexatrien-1-one (**2a**) based on 2D NMR data. In order to further support the structure, this work reports the single-crystal X-ray analysis, the complete ^{1}H NMR assignment by full spin−spin simulation, and the absolute configuration of the diacetate **2b** derived via vibrational circular dicroism measurements in comparison with density functional theory calculated data.



The patented structure **1** of rosmaridiphenol,[1] an antioxidant isolated from popularly used rosemary,[2] *Rosmarinus officinalis* L. (Lamiaceae), was recently revised on the basis of 2D NMR data to structure **2a**.[3] Due to the wide use of rosemary as reported in Wikipedia, it is imperative that the structure for rosmaridiphenol is correct. Additional evidence to support the structure **2a** was deemed necessary, and thus the preparation of rosmaridiphenol diacetate (**2b**) was carried out for this purpose. Herein, we provide single-crystal X-ray data, absolute configuration determination by vibrational circular dicroism (VCD), and full ^{1}H NMR assignment using spin−spin simulation in support of structure **2b**.

The relative configuration of **2a**, established by NMR techniques,[3] was confirmed by X-ray crystal analysis of diacetylated analogue **2b**. According to the X-ray results (Figure 1), the cyclohexanone and cycloheptene rings are in chair conformations, whereas the orientation of the two acetyl groups is *anti* to one another. Additionally, the orientation of the isopropyl group is defined by the C-12−C-13−C-15−H-15 torsion angle of −16.9°, placing the methyl groups far from one of the acetyl groups. The crystal data for **2b** are summarized in the Experimental Section, and the fractional atom coordinates are given in the Supporting Information.

The absolute configuration of **2b** was deduced by comparison of the experimental VCD spectrum with the calculated spectrum, generated using density functional theory[4] (DFT), with the experimental VCD spectrum. This methodology has proven useful for absolute configuration assignment of natural products.[5] The molecular model was constructed using the single-crystal X-ray atom coordinates and subjected to a full minimization routine employing molecular mechanics (MMFF) using the Spartan'04 package (Wavefunction, Irvine, CA, USA). The energy value was monitored as the convergence criterion to yield the global minimum energy value of 80.3 kcal/mol. This conformer was used as the starting point in a Monte Carlo search, which afforded 52 conformations within a 10 kcal/mol

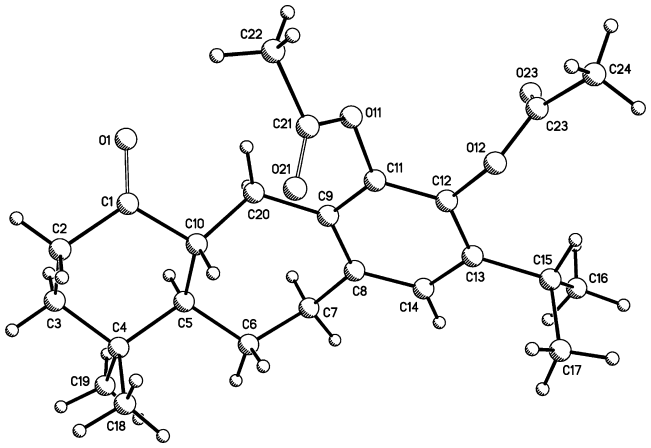


Figure 1. Single-crystal X-ray structure of **2b**.

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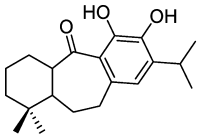

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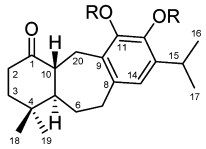

2a R = H
2b R = OAc

energy window. DFT single-point energy values of all conformations were calculated at the B3LYP/DGDZVP level of theory, using the Gaussian'03W program (Gaussian Inc., Pittsburgh, PA, USA), which afforded only 11 conformations in a 4 kcal/mol range, accounting for 99.7% of the corresponding Boltzmann distribution. Geometry optimization followed by vibrational calculations afforded free energy values for these 11 conformers, which in turn showed that only the five conformers **I**–**V** of **2b** (Table 1) represent 99.3% of the conformational distribution (Figure 2). These five conformations showed the same disposition of the polycyclic atoms and only differ in the orientations of the isopropyl and acetate groups. The vibrational calculations also produced IR and VCD intensities for each of the five selected conformations, which were used to obtain the corresponding Boltzmann-weighted spectra according to the free energy derived populations. Computer comparison of the calculated VCD and IR frequencies, using the CompareVOA (BioTools Co., Jupiter, FL, USA) software, allowed calculation of an anharmonicity (anH) factor of 0.973 and an enantiomeric similarity index (ESI) of 58.3. The good agreement (Figure 3) between experimental and theoretical VCD spectra permits the absolute configuration assignment of **2b** as (5*S*,10*R*)-11,12-diacetoxy-8,11,13-icetexatrien-1-one, which directly correlates with natural product **2a**.

The experimental ^{1}H NMR spectrum of **2b** (lower trace, Figure 4) exhibited the presence of the isopropyl group, the aromatic proton, and the geminal methyl groups for the icetexane skeleton at chemical shifts very close to those described for natural product **2a**.[3] Additionally, the methyl groups of two *O*-acetyl groups resonated at δ 2.29 and 2.30. A gHSQC experiment was used to identify the hydrogen atoms on each methylene group. Thus, the methylene hydrogens α to the carbonyl group (Figure 5) showed a doublet of doublets of

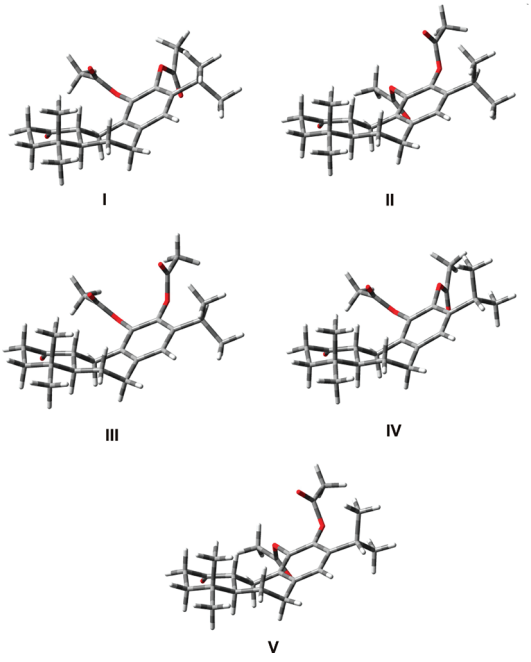

Figure 2. Minimum energy DFT structures of **2b**.

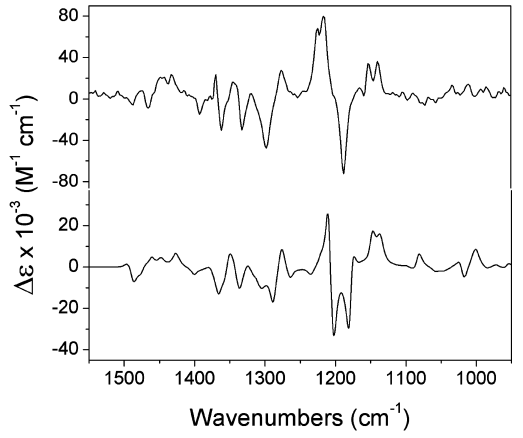

Figure 3. Experimental (top) and calculated (bottom) VCD spectra of **2b**.

doublets at δ 2.50 and a signal overlapped with one acetyl group at δ 2.30, both correlating with C-2 at δ 38.2. The vicinal coupling constant predicted by the Altona equation,[6] in agreement with the presence of two large (J_{gem} and J_{2ax3ax}) and one small coupling constant (J_{2ax3eq}) in the spin system at δ

Table 1. Molecular Mechanics Relative Energies, Molecular Mechanics Abundances, and DFT Thermochemical Parameters and Abundances of the Five More Stable Conformers of Rosmaridiphenol Diacetate (2b)[a]

conformer of **2b**	ΔE_{MMFF}[b]	p_{MMFF}[c]	ΔE_{sp}[d]	p_{sp}[c]	ΔH_{298}[e,f,g]	$p_{\Delta H}$[c]	ΔG_{298}[e,f]	$p_{\Delta G}$[h]
I	0.00	72.4	0.00	56.6	0.00	68.2	0.00	55.6
II	1.13	10.7	1.42	5.1	0.89	15.2	0.22	38.5
III	1.63	4.6	0.53	23.2	1.29	7.8	1.67	3.3
IV	1.18	9.9	1.32	6.0	1.33	7.2	2.19	1.4
V	2.22	1.7	2.61	0.7	2.24	1.6	2.27	1.2

[a]Conformers are ordered according to their DFT free energy relative abundances. [b]Molecular mechanics energy of conformers obtained from a Monte Carlo stochastic search, in kcal/mol relative to **2b-I** with E_{MMFF} = 80.34 kcal/mol. [c]Population in % calculated from molecular mechanics energies, DFT single-point energies, and DFT enthalpies. [d]Single-point energies relative to conformer **2b-I** with E_{sp} = −1309.987 492 au. [e]Thermal enthalpies (ΔH_{298}) and thermal free energies (ΔG_{298}) in kcal/mol relative to conformer **2b-I** and calculated at 298 K and 1 atm. [f]For conformer **2b-I** the absolute values are H_{298} = −1309.590 144 and G_{298} = −1309.683 231 au. [g]ΔE_{298} values equal to ΔH_{298} in all conformations. [h]Final DFT population in % calculated from ΔG_{298} values.

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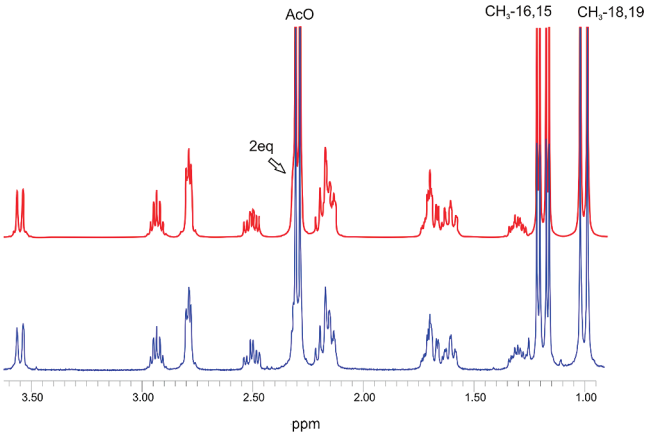

Figure 4. Full ^{1}H NMR spin−spin analysis by PERCH iteration at 500 MHz of **2b**. The lower trace is the experimental and the upper trace the spin−spin simulated spectrum.

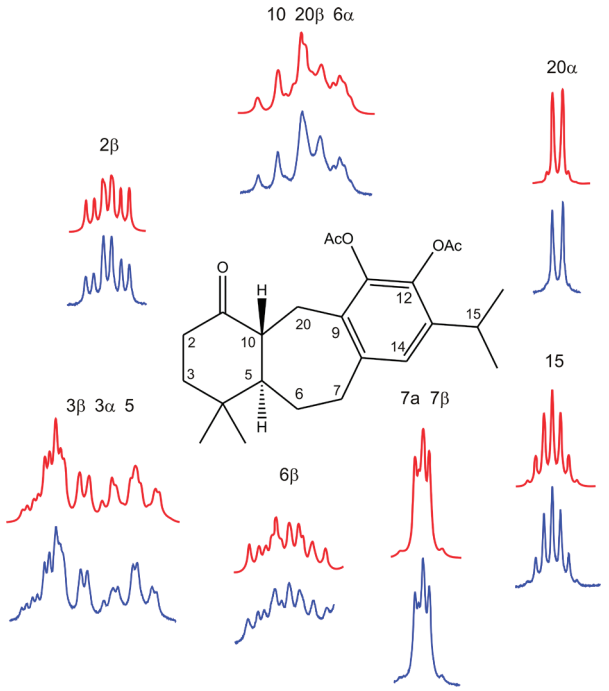

Figure 5. Individual comparison of experimental (blue) and calculated (red) plots for some hydrogens of **2b**.

2.50 (Table 2), allows its assignment to the axial C-2 hydrogen. Consequently the overlapped signal at δ 2.30 is assigned to the equatorial hydrogen. Similarly, for the pair of C-6 hydrogens two multiplets at δ 2.15 and 1.30 were associated with the carbon at δ 28.9. The low-frequency signal has one small (J_{6ax7eq}) and three large (J_{gem}, J_{6ax7ax}, J_{6ax5ax}) coupling constants and corresponds to the axial hydrogen. In the same sense, the doublet of doubles at δ 3.50 showing one large (J_{gem}) and one small ($J_{20eq10ax}$) coupling constant was assigned to the equatorial hydrogen at C-20 (δ 25.5) and the overlapped signal at δ 2.19 to the axial hydrogen. For the remaining C-3 and C-7 methylene groups, the identification of axial and equatorial hydrogens was not immediately evident due to signal overlap. For C-3 two multiplets around δ 1.70 show correlation with the carbon atom at δ 41.8, and for C-7 two doublets of doublets of doublets at δ 2.79 were identified for the carbon at δ 34.6. Finally, inspection of the methine hydrogens reveals the signals at δ 1.61 and 2.16 as multiplets due to coupling of axial hydrogens at C-5 (δ 57.4) and C-10 (δ 49.7), respectively, and the adjacent methylene hydrogens.

The ^{1}H NMR spin−spin analysis of **2b** was performed using the PERCH software (Perch Solutions, Kuopio, Finland). This methodology allows analysis of complex high-resolution ^{1}H NMR spectra, through iterative minimization of the simulated and experimental spectra differences.[7] Thus, the analysis determines chemical shifts and coupling constants for all hydrogen atoms in a molecule and has been recently applied for the complete ^{1}H NMR assignments of some natural products.[8]

The free induction decay data for **2b** at 500 MHz were used for the preparation of the experimental frequency-dependent spectrum, while the minimum energy DFT//B3LYP/DGDZVP conformer **I** (Figure 2) was used for the prediction and generation of the theoretical spectrum in the PERCH suite. Before iteration, chemical shift values from the experimental spectrum and vicinal coupling constants derived from Altona estimation were incorporated in the parameter table of the editor module (PMS) in order to get a good calculated spectrum approximation. This spectrum was submitted to iteration using the total-line-shape-fitting method (T-mode) in the PERCH iterator. The observed and simulated spectra showed good agreement, with a root-mean-square deviation of 0.060 Hz, providing chemical shifts and coupling constants with a high degree of accuracy. The results for the ^{1}H NMR full spin−spin analysis of **2b** are summarized in Table 2 and Figures 4 and 5. Although the PERCH calculation provides the chemical shifts with six significant figures, the experimental 500 MHz spectrum was acquired with a resolution better than 0.4 Hz, and therefore chemical shifts with three digits after the decimal point constitute a proper description. Table 2 reports PERCH-derived J values with two figures after the decimal point, as in similar cases.[8a] The full spin−spin analysis permitted assignment of all coupling constants and chemical shift values. Even though H-2$_{eq}$ appears as an overlapped signal with one acetyl group, the spin−spin simulation procedure permits determination of all spectroscopic values since this is the only overlapped signal.

In conclusion, the additional information given in the present work provides convincing evidence for the structural assignment of rosmaridiphenol diacetate as **2b**.

EXPERMENTAL SECTION

General Experimental Procedures. The melting point was determined on a Fisher-Johns melting point apparatus and is uncorrected. Optical rotations were determined in $CHCl_3$ on a Perkin-Elmer 341 polarimeter. The UV spectrum was determined on a Perkin-Elmer Lambda 12 UV/vis spectrophotometer. The VCD and IR spectra were acquired on a BioTools Dual PEM Chiral*IR* FT-VCD spectrophotometer. The 500 MHz ^{1}H NMR spectrum was measured on a Varian system 500 in $CDCl_3$ solution containing TMS as the internal standard at room temperature (22 °C). The 500 MHz data were acquired with a digital resolution of 0.14 Hz/point and a spectral width of 9.38 kHz. Fourier transformation was applied with zero filling to 64k points. The ^{13}C NMR spectrum and gHSQC measurement were performed on a Varian Mercury 300 spectrometer using $CDCl_3$ solutions containing TMS as the internal standard. The LR-MS was recorded on an AB SCIEX 3200 Q TRAP mass spectrometer. Column chromatography was carried out on Merck silica gel 60 (Aldrich, 230−400 mesh).

Rosmaridiphenol Diacetate (2b). A solution of **2a** (80 mg, 0.253 mmol) in pyridine (2 mL) was cooled in an ice bath, and acetic anhydride (1 mL) was added while stirring. The solution was kept at room temperature for 24 h and cooled in an ice-bath, and after addition of water (2 mL) the reaction mixture was extracted with

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Table 2. Chemical Shifts, Coupling Orders, and *J*-Values of 2b Determined by Spectroscopic Iteration Using the PERCH Software[a]

atom	δ	multiplicity	J	J_{altona}	dihedral angle
H-2_{eq}	2.301	ddd	J_{gem} = −13.05, J_{3ax} = 5.72, J_{3eq} = 2.38	J_{3ax} = 4.1, J_{3eq} = 3.1	Φ_{3ax} = 55.4, Φ_{3eq} = −60.3
H-2_{ax}	2.504	ddd	J_{3ax} = 15.33, J_{gem} = −13.05, J_{3eq} = 5.93	J_{3ax} = 13.9, J_{3eq} = 3.2	Φ_{3ax} = 175.0, Φ_{3eq} = 59.3
H-3_{ax}	1.672	ddd	J_{2ax} = 15.33, J_{gem} = −13.01, J_{2eq} = 5.72	J_{2ax} = 13.9, J_{2eq} = 4.1	Φ_{2ax} = 175.0, Φ_{2eq} = 55.4
H-3_{eq}	1.712	ddd	J_{gem} = −13.01, J_{2ax} = 5.93, J_{2eq} = 2.38	J_{2ax} = 3.2, J_{2eq} = 3.1	Φ_{2eq} = −60.3, Φ_{3eq} = 59.3
H-5	1.606	ddd	J_{10} = 15.12, J_{6ax} = 10.90, J_{6eq} = 2.47	J_{10} = 12.9, J_{6ax} = 12.4, J_{6eq} = 2.4	Φ_{10} = −177.1, Φ_{6ax} = 179.0, Φ_{6eq} = −65.6
H-6_{eq}	2.148	dddd	J_{gem} = −13.47, J_{7eq} = 7.89, J_5 = 2.47, J_{7ax} = 1.15	J_{7eq} = 7.7, J_{7ax} = 0.7, J_5 = 2.4	Φ_{7eq} = −36.8, Φ_{7ax} = 78.3, Φ_5 = −65.6
H-6_{ax}	1.305	dddd	J_{gem} = −13.47, J_{7ax} = 11.69, J_5 = 10.90, J_{7eq} = 1.21	J_{7ax} = 12.9, J_{7eq} = 0.8, J_5 = 12.4	Φ_5 = 179.0, Φ_{7ax} = −167.5, Φ_{7eq} = 77.4
H-7_{ax}	2.792	ddd	J_{gem} = −14.44, J_{6ax} = 11.69, J_{6eq} = 1.15	J_{6ax} = 12.9, J_{6eq} = 0.7	Φ_{6ax} = −167.5, Φ_{6eq} = 78.3
H-7_{eq}	2.788	ddd	J_{gem} = −14.44, J_{6eq} = 7.89, J_{6ax} = 1.21	J_{6eq} = 7.7, J_{6ax} = 0.8,	Φ_{6eq} = −36.8, Φ_{6ax} = 77.4
H-10	2.188	ddd	J_5 = 15.12, J_{20eq} = 1.92, J_{20ax} = 9.88	J_5 = 12.9, J_{20eq} = 1.0, J_{20ax} = 11.0	Φ_5 = −177.1 Φ_{20eq} = −85.8, Φ_{20ax} = 160.1
H-14	6.924	s			
H-15	2.934	qq	J_{16} = 6.90, J_{17} = 6.90		
CH_3-16	1.209	d	J_{15} = 6.90		
CH_3-17	1.168	d	J_{15} = 6.90		
CH_3-18	1.020	s			
CH_3-19	0.988	s			
H-20_{ax}	2.159	dd	J_{gem} = −14.79, J_{10} = 9.88	J_{10} = 11.0	Φ_{10} = 160.1
H-20_{eq}	3.551	dd	J_{gem} = −14.79, J_{10} = 1.92	J_{10} = 1.0	Φ_{10} = −85.8
AcO	2.306	s			
AcO	2.286	s			

[a]Dihedral angles and vicinal *J*-values determined using the Altona equation are also shown.

EtOAc (3 × 20 mL). The combined organic extract was washed with 10% HCl (3 × 20 mL), water (20 mL), a 5% solution of $KHCO_3$ (1 × 20 mL), and water again, dried over anhydrous Na_2SO_4, filtered, and evaporated on a rotary evaporator. The residue was purified by flash column chromatography. Elution with hexanes/EtOAc (4:1) afforded the title compound (77 mg, 76%), and subsequent recrystallization from MeOH gave colorless needles; mp 158−160 °C; $[\alpha]_{589}$ +88.0, $[\alpha]_{578}$ +92.6, $[\alpha]_{546}$ +106.0, $[\alpha]_{436}$ +193.2 (*c* 0.5, $CHCl_3$); UV (EtOH) λ_{max}(log ε) 201.2 (4.6) and 216.7 (3.8) nm; IR ($CDCl_3$) ν_{max} 1764, 1704, 1210, 1185 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.0 (C-18, CH_3), 20.3 (OAc, CH_3), 20.4 (OAc, CH_3), 22.8 (iPr, CH_3), 23.0 (iPr, CH_3), 25.5 (C-20, CH_2), 27.4 (C-15, CH), 28.9 (C-6, CH_2), 29.4 (C-19, CH_3), 34.1 (C-4,C), 34.6 (C-7, CH_2), 38.2 (C-2, CH_2), 41.8 (C-3, CH_2), 49.7 (C-10, CH), 57.4 (C-5, CH), 123.1 (C-14, CH), 131.3 (C-8, C), 138.1 (C-13, C), 138.9 (C-12, C), 139.9 (C-11, C); 142.5 (C-9, C), 168.7 (OAc, CO), 168.8 (OAc, CO); 211.5 (C-1); EIMS *m*/*z* 400 (M^+ + 1), 341 (100), 95 (24): HREIMS *m*/*z* 401.2323 (M^+ + H) (calcd for $C_{24}H_{32}O_5$ + H, 401.2328).

Single-Crystal X-ray Analysis of Rosmaridiphenol Diacetate (2b). A crystal measuring 0.38 × 0.36 × 0.34 mm was mounted on a Nonius Bruker CAD4 diffractometer equipped with graphite-monochromated Cu Kα radiation, λ = 1.54184 Å. The crystal was monoclinic, space group $P2_1$, with cell dimensions *a* = 8.130(1) Å, *b* = 13.484(3) Å, *c* = 10.230(2) Å, β = 92.60(4)°, *V* = 1120.4(4) $Å^3$, ρ_{calc} = 1.187 g/cm^3 for *Z* = 2, $C_{24}H_{32}O_5$, MW = 400.50, and *F*(000) = 432 e. The structure was solved by direct methods using SHELX97. For the structural refinement, the non-hydrogen atoms were refined anisotropically, and the hydrogen atoms were refined isotropically. A total of 1905 reflections were collected within a θ range of 5.45−59.95° for $-9 \le h \le 9$, $0 \le k \le 14$, $0 \le l \le 11$. The unique reflections were 1713, the observed reflections were 1688, and final discrepancy indices, refining 285 parameters, were R_F = 3.4% and R_w = 9.6%. The final difference Fourier map was essentially featureless, the highest residual peaks having a density of 0.151 e/$Å^3$. Crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre under CCDC deposition number 867863. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 IEZ, UK. Fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.

VCD Analysis and Calculations. A solution of **2b** (5.2 mg) in 150 μL of $CDCl_3$ was placed in a BaF_2 cell with a path length of 100 μm, and data were acquired at a resolution of 4 cm^{-1} during 3 h. Geometry optimizations for rosmaridiphenol diacetate (**2b**) were carried out using the MMX force field[9] calculations in the Spartan'04W package (Wavefunction, Irvine, CA, USA). The conformational search was made with the Monte Carlo protocol[10] obtaining a total of 52 conformers in a 10 kcal/mol energy window. These conformations where subjected to single-point energy calculations at the DFT//B3LYP/DGDZVP level of theory, affording 11 relevant conformers in a 4 kcal/mol range and accounting for 99.7% of the total Boltzmann population. The geometries of these 11 DFT conformers were optimized at the B3LYP/DGDZVP level of theory, and the corresponding free energies were calculated using the same level of theory, which were used to produce a more accurate Boltzmann distribution, showing that only five conformations represented 99.3% of the total population. Conformers **I**−**V** preserve the six- and seven-membered ring in chair conformations with changes in rotation of the two acetyl groups. The vibrational frequencies (IR and VCD), dipole strengths, and rotational strengths for these five conformations, also obtained in the same calculations, were used to produce a calculated VCD spectrum obtained by weighing the individual spectra according to the free energy Boltzmann distribution. This spectrum was compared with the experimental VCD spectrum after application of an anharmonicity correction factor of 0.973, which was estimated *in silico* using the CompareVOA software[11] (BioTools, Inc., Jupiter, FL, USA, 2010). All DFT calculations were performed with the Gaussian 03W program (Gaussian Inc., Pittsburgh, PA, USA). For calculations of vicinal coupling constants, the dihedral angles of conformer **I** of **2b** (Figure 2) were used in the Altona equation.[6]

^1H NMR Full Spin−Spin Analysis. The spectroscopic analysis of **2b** was performed using the PERCH software (PERCH Solutions Ltd., Kuopio, Finland). The ^1H NMR experimental data at 500 MHz were edited in the preparation module (PAC) of the PERCH shell. The minimum energy DFT//B3LYP/DGDZVP conformer **I** of **2b** was imported to the editor structure module for prediction of a calculated spectrum. Before iteration of the calculated spectrum, the known coupling constant values for **2b** in the parameter table of the editor module (PMS) were incorporated, as well as some vicinal coupling constants obtained from the Altona estimation. Thus, the calculated spectrum was submitted to iteration using the total-line-shape-fitting

mode from the PERCH iterator. The root-mean-square deviation was 0.060 Hz after 30 iteration cycles.

■ ASSOCIATED CONTENT

Supporting Information

X-ray fractional atom coordinates for **2b**. Atom coordinates for low-energy conformers **I**–**V** of **2b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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