Absolute configuration assignment of an unusual homoisoflavanone from *Polygonum ferrugineum* using a combination of chiroptical methods

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A B S T R A C T

The absolute configuration of the homoisoflavanone 5,7-dihydroxy-6-methoxy-3-(9-hydroxy-phenyl-methyl)-chroman-4-one (**1**), found in *Polygonum* species, has been determined as 3*R*,9*R* by using a combination of electronic and vibrational chiroptical spectroscopic methods. The experimental spectra were interpreted with the aid of density functional theory calculations. The risks of relying upon a single method for stereochemical characterization of natural products are discussed.

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Polygonaceae is a cosmopolitan plant family widely distributed in temperate regions and sometimes in the tropics. The genus *Polygonum* comprises about 300 species, many of them being edible or bearing medicinal properties.[1] It is well known for producing a variety of secondary metabolites including sesquiterpenoids,[2] triterpenoids,[3] anthraquinones,[4] phenylpropanoids,[5] lignans and flavonoids,[6] coumarins,[7,8] stilbenoids,[9,10] and tannins.[11]

In 2006, López et al.[12] reported for the first time the isolation of the unusual homoisoflavanone 5,7-dihydroxy-6-methoxy-3-(9-hy-droxy-phenylmethyl)-chroman-4-one (**1**) from dry leaves of *Polygonum ferrugineum* (Polygonaceae). One year later, Midiwo et al.[13] reported the same structure from *Polygonum senegalense* and claimed it to be a novel compound. Interestingly, López reported a specific rotation value at 589.3 nm (sodium D line) of -8.67 deg[dm g/cm^3]$^{-1}$ in dichloromethane for **1** while Midiwo found +41 in methanol. As none of the previous reports assigned the absolute configuration of this compound, one might infer that *P. ferrugineum* and *P. senegalense* produce enantiomeric homoisoflavanones. Additionally, in 2012, Dzoyem et al.[14] reported

the same homoisoflavanone from *Polygonum limbatum* as a racemate ([α]$_D$ 0 in methanol). Therefore, in order to unambiguously determine the absolute stereostructure of **1**, and consequently clarify the biosynthetic relationship between these species, we decided to apply a combination of different chiroptical methods and density functional theory calculations to the homoisoflavanone **1** isolated from *P. ferrugineum*.

A recent survey[15] revealed the evolution in the use of chiroptical methods for stereochemical characterization of natural products over the last fifteen years, especially the use of vibrational circular dichroism (VCD) and Raman optical activity (ROA). Nevertheless, despite the widespread occurrence of flavonoids in nature very few reports dealt with the application of vibrational optical activity (VOA) methods for stereochemical characterization of this class of secondary metabolites. This will be the first example of the use of VCD and ROA for homoisoflavanones.

Based on the striking difference for the optical rotation of the homoisoflavanones isolated from *P. ferrugineum* and *P. senegalense*, the first approach to assess their stereochemical properties was to measure the specific rotation of **1** from *P. ferrugineum* in methanol, dichloromethane, and chloroform. The results in methanol ([α]$_D$ +62; *c* 0.1) and in dichloromethane ([α]$_D$ −13.5; *c* 0.2) were in accordance with the literature values, despite small discrepancies, and suggest that *P. ferrugineum* and *P. senegalense* produce

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compound **1** with the same absolute configuration. Interestingly, the specific rotation of **1** in chloroform was $[\alpha]_D$ +44 (c 0.05, CHCl₃), which is much closer to the value obtained in methanol than to that measured in dichloromethane. This fact demonstrates the risks of comparing optical rotation values in different conditions, even when apparently similar solvents are used. In this case, the best approach would be the measurement of optical rotation at different wavelengths, known as optical rotatory dispersion.

Calculations of the optical rotations at a single wavelength for compound **1** using quantum chemical methods were not carried out due to the great uncertainty arising in such calculations for systems with multiple electronic transitions.[16] Additionally, the variations observed in the sign of optical rotation in different solvents for compound **1** may require molecular modeling with inclusion of explicit solvation, which is more time consuming. Thus, in order to determine the absolute configuration of **1** a combination of electronic circular dichroism (ECD), VCD, and ROA was used with the aid of density functional theory (DFT) predictions of theoretical spectra. In 2006 López et al. reported ECD values for **1** in methanol, however, no conclusion was drawn from the data. The authors then suggested that ECD calculations for all diastereoisomers possible for **1** would be necessary to solve its absolute stereochemistry. Therefore, molecules with 3S,9S and 3S,9R configuration were arbitrarily chosen for conformational searches, geometry optimizations and ECD, VCD, and ROA spectral calculations. The chiroptical properties for their enantiomers were obtained by multiplying the theoretical spectra by (−1). From the optimized geometries, it was possible to observe that only the enantiomeric pair 3S,9S/3R,9R (Fig. 1) would allow for the coupling constant observed for H-3 and H-9 (J < 10 Hz), and also for the easy elimination of the OH at C-9 under mild conditions (pyr, rt, E₂ mechanism, **Conf1–2**) to yield an E-configured product. The first technique used to assess the absolute configuration on **1** was ECD. The good agreement between experimental and calculated data at the B3PW91/PCM(MeOH)/TZVP level for (3R,9R)-**1** (3S,9S spectrum multiplied by −1) suggested the absolute configuration of **1** to be 3R,9R (Fig. 1). Nevertheless, the theoretical calculations revealed that ECD was not sensitive to the absolute configuration of the chiral center at C-9, since molecules with 3R,9R and 3R,9S yielded very similar spectra (Fig. 1). The analysis of the molecular orbitals involved in the main electronic transitions observed in the ECD spectra of both (3R,9R)- and (3R,9S)-**1** revealed that they are dominated by π–π^*, n–π^*, and charge transfer-type transitions between the two aromatic rings. In spite of the opposite configuration at C-9, the relative positioning of the chromophores was maintained

in the two epimers (see Supplementary material). Therefore, we decided to use VOA methods to assign unambiguously the absolute configuration of **1**. The relatively good agreement between experimental and calculated [B3PW91/PCM(DMSO)/6-31+G(d,p)] VCD spectra for the Boltzmann average of the four lowest-energy conformers of **1** confirmed the conclusion obtained from the ECD study (Fig. 2). The quality of the experimental data was affected
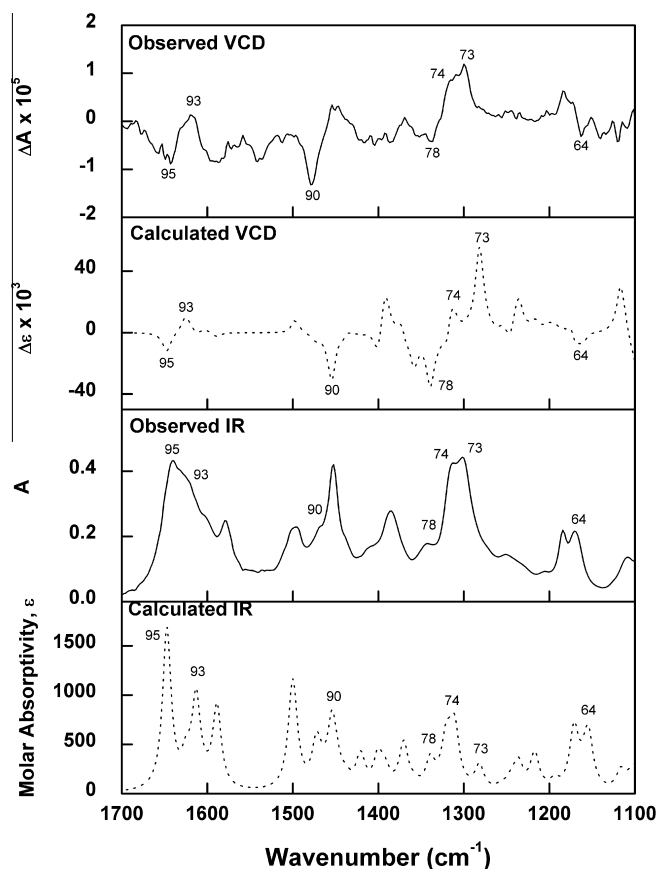

Figure 2. Comparison of the IR and VCD spectra of experimental (−)-**1** with the calculated [B3PW91/6-31+G(d,p) in DMSO using PCM] IR and VCD spectra of the Boltzmann average of the four lowest-energy conformers of the corresponding (3R,9R)-**1**. The comparison establishes the absolute configuration of this molecule as (−)-(3R,9R)-**1**. Numbers represent selected fundamental vibrational modes.
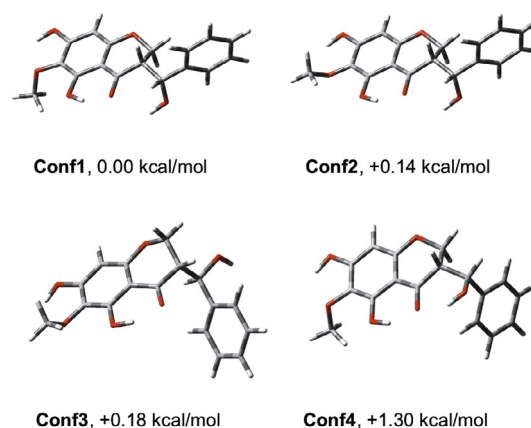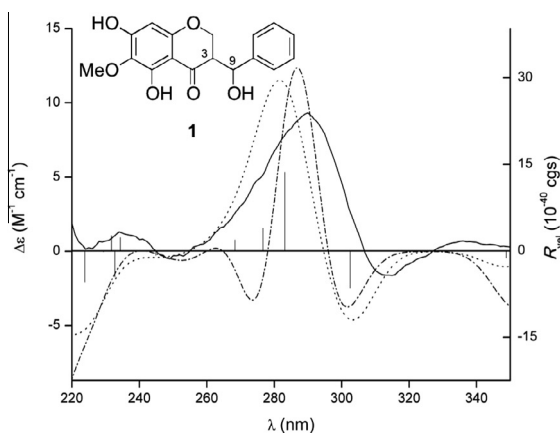

Figure 1. (Left) Comparison of the ECD spectrum of experimental (−)-**1** (solid line) with the calculated [B3PW91/TZVP//B3PW91/6-31+G(d,p) in methanol using PCM] ECD spectra of the Boltzmann average of the lowest-energy conformers of the corresponding (3R,9R)-**1** (dotted line) and (3R,9S)-**1** (dash-dotted line). Bars represent rotational strengths for the weighted ECD spectra of (3R,9R)-**1**. (Right) Structure and relative energy of the four lowest-energy conformers of (3R,9R)-**1** used for ECD calculations.
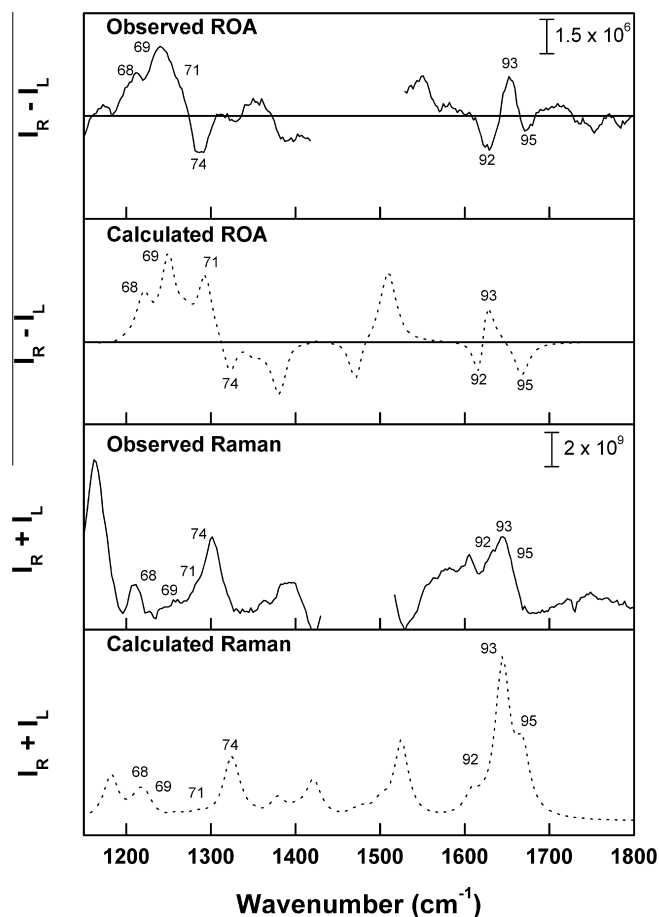
Figure 3. Comparison of the Raman and ROA spectra of experimental (−)-**1** with the calculated [B3LYP/TZVP//B3LYP/TZVP in MeOH using PCM] Raman and ROA spectra of the lowest-energy conformer of the corresponding (3*R*,9*R*)-**1**. The comparison establishes the absolute configuration as (−)-(3*R*,9*R*)-**1**. Deleted spectral regions correspond to overlapping signals from methanol CH$_3$ deformation modes. I_R and I_L are the intensities of scattered right- and left-circularly polarized light, respectively. Numbers represent selected fundamental vibrational modes.

by the low solubility of **1** in DMSO-d_6, which was needed in order to avoid isotopic exchange commonly observed in MeOH-d_4. In spite of that, the main bands present in the VCD spectrum of **1** arise from rather localized normal modes involving the chiral centers at C-3 and C-9, thus providing a better discriminative power for diastereoisomers compared to that of ECD. The bands at 1299 cm^{-1} (positive, fundamental 73) and 1346 cm^{-1} (negative, fundamental 78) arise from C–H bendings and C–C stretches; the negative band at 1479 cm^{-1} (fundamental 90) involves the scissoring vibration of the methylene group at C-2 and C–C stretches within the chromanone moiety. The degree of agreement between experimental and calculated IR/VCD spectra was assessed using the confidence level algorithm[17] and resulted in an enantiomeric similarity index (ESI) of 53.4 and a confidence level of 85% for the 3*R*,9*R* configuration. Finally, the comparison of calculated (one-step[18] B3LYP/TZVP) and experimental ROA spectra (Fig. 3) for **1** confirmed its absolute configuration as 3*R*,9*R* and also indicated a different conformer composition in methanol when compared to the DMSO solution. The bands at 1250 and 1290 cm^{-1} (in-plane and out-of-plane C–H bending of C-3 and C-9; and CH$_2$ twisting, fundamentals 69 and 74) as well as the −,+,− pattern

centered 1650 cm^{-1} (C=O stretch and C=C deformations of rings A and B, fundamentals 92, 93, and 95) were only observed for **Conf4**. This finding suggests that in methanol **1** adopts this conformation predominantly. The output of the confidence level algorithm for this assignment was as follows: ESI = 64.1 and confidence level of 91% for the 3*R*,9*R* configuration.

Therefore, the absolute configuration of the homoisoflavanone (−)$_{DCM}$-**1** was unambiguously assigned as 3*R*,9*R* by means of a combination of chiroptical methods and theoretical calculations. The risks of comparing optical rotation values in different conditions have been clearly demonstrated. VCD and ROA were superior to ECD in distinguishing diastereoisomers, and the former techniques were complementary to each other in probing conformational preferences of **1** in different solvents. Finally, *P. ferrugineum* and *P. senegalense* seem to biosynthesize homoisoflavanones with the same stereochemical properties, while *P. limbatum* seems to produce both enantiomers non-stereospecifically.

Supplementary data

Supplementary data (experimental procedures, computational methods, lowest-energy conformers, IR/VCD and Raman/ROA spectra of (3*R*,9*S*)-**1**; molecular orbitals of (3*S*,9*S*)-**1**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.09.102.

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