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Absolute configuration assignment of caffeic acid ester derivatives from *Tithonia diversifolia* by vibrational circular dichroism: the pitfalls of deuteration

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ABSTRACT

Recently, it was observed that infrared (IR) and vibrational circular dichroism (VCD) calculations including deuterated hydroxyl groups in phenolic and saccharide moieties improved significantly the agreement with experimental data obtained in methanol-d_4. In the present study, the relative and absolute configurations of three methanol-soluble caffeic acid ester derivatives **1**–**3**, isolated from *Tithonia diversifolia*, were established by a combined use of experimental and calculated ^{13}C NMR chemical shifts, as well as electronic circular dichroism (ECD) and VCD spectroscopies. Interestingly, the attempt to reproduce the deuteration pattern arising from possible isotopic exchange in methanol-d_4 solution led to nearly mirror image calculated VCD spectra for **1** when compared to the non-deuterated molecule with the same absolute configuration. This latter fact can potentially lead to absolute configuration misassignments. A closer inspection of the vibrational chiroptical properties of **1** revealed that the deuteration status of the tertiary hydroxyl group at C-2 is critical for the correct reproduction of experimental VCD data in protic solvents. Therefore, in the case of stereochemical analysis of polar chiral natural product molecules, a combination of VCD and ECD is recommended.

1. Introduction

Tithonia diversifolia (Helms.) A. Gray, commonly known as tree marigold or Mexican sunflower, is a perennial herb from the family Asteraceae, native to both Mexico and Central America. Two reports have demonstrated that this species constitutes a source of caffeic acid ester derivatives, secondary metabolites with reported antioxidant, antimicrobial and anti-inflammatory activities.[1,2] In a recent study, the influence of the environment on the secondary metabolite composition of *T. diversifolia* was assessed by LC–MS based metabolomics,[3] where several caffeic acid ester derivatives were identified, while other mass patterns did not return known hits in our *in-house* database (www.asterbiochem.org/asterdb). Herein, isolation procedures were conducted for their accurate identification by 1D- and 2D-NMR spectroscopy that resulted on the structural elucidation of a new caffeic acid ester derivative **2** and two previously isolated but incompletely assigned compounds **1** and **3**. The relative and absolute configurations of **1**–**3** (Fig. 1) were then determined by combining experimental and calculated ^{13}C NMR chemical shifts, ECD and VCD spectroscopies.

VCD, which arises from the differential absorption of left- and right- circularly polarized light by a chiral molecule during a vibrational transition, has become one of the methods of choice for conformational and configurational analyses of natural products.[4,5] Recently, it has been demonstrated that IR and VCD calculations including partially deuterated hydroxyl groups in ellagitannins improved significantly the agreement with experimental data obtained in methanol-d_4.[6] Therefore, taking into account that the VCD spectra obtained for compounds **1**–**3** were recorded in methanol-d_4, in this work we also investigated theoretically the effects of different deuteration patterns on the IR and VCD properties of these compounds. Interestingly, compound **1** was the most sensitive to varying degrees of deuteration with striking differences between deuterated and non-deuterated molecules. All of these results indicate a complex deuteration scenario in solution, which may lead to absolute configuration misassignments.

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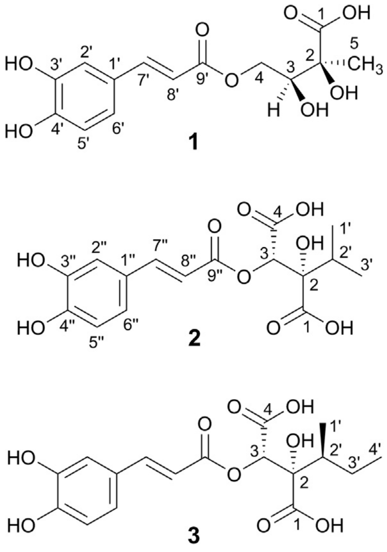

Figure 1. Structures of compounds **1**–**3**.

2. Results and discussion

The HRESIMS of **1** showed a *pseudo*-molecular ion peak [M−H]$^-$ at *m*/*z* 311.0762, consistent with the molecular formula $C_{14}H_{16}O_8$ (calcd for $C_{14}H_{15}O_8$, 311.0767). The 1D- and 2D-NMR data of **1** resembled those previously reported for 4-*O*-caffeoyl-2-*C*-methyl-D-threonic acid.[7] However, herein, the relative configuration of **1** was inferred to be *erythro* because the NOE interaction between H-3 and H_3-5. Thus, the structure of **1** was assigned as 4-*O*-caffeoyl-2-*C*-methyl-D-erythronic acid.

In order to verify the relative configuration proposed for **1**, compound **1a** was obtained by translactonization of **1** (Fig. 2).[7,8] The HRESIMS of **1a** showed a *pseudo*-molecular ion peak [M−H]$^-$ at *m*/*z* 293.0667, consistent with the molecular formula of $C_{14}H_{14}O_7$ (calculated for $C_{14}H_{13}O_7$, 293.0661). The 1D- and 2D-NMR data of **1a** were in agreement with those from the previously described compounds 3-*O*-caffeoyl-2-*C*-methyl-D-erythrono-1,4-lactone[8] and 3-*O*-caffeoyl-2-*C*-methyl-D-threono-1,4-lactone.[7] The NOE interaction between H-3 and H_3-5 in **1a** further supported the *erythro* relative configuration of **1a**, and therefore **1**.

The HRESIMS of **2** showed a *pseudo*-molecular ion peak [M−H]$^-$ at *m*/*z* 353.0880, consistent with the molecular formula $C_{16}H_{18}O_9$ (calculated for $C_{16}H_{17}O_9$, 353.0872). The ^{1}H and ^{13}C NMR spectra of **2** displayed signals corresponding to the caffeoyl and a butanedioic acid moieties, resembling the known compound **3** (see below). However, compound **2** contains a 2-propyl instead of the 2-butyl side chain present in **3**, supported by the ^{1}H NMR patterns at δ_H 2.32 (sept, $J = 6.8$, H-2′), 1.04 (d, $J = 6.8$, H_3-1′) and 0.98 (d, $J = 6.8$, H_3-3′). The cross peaks from the protons of the 2-propyl side chain (H_3-1′, H-2′ and H_3-3′) to C-1 and C-2 in the HMBC spectrum of **2** confirmed the position of the 2-propyl group at C-2. The long-range correlations between H-3 and C-1, C-2, C-4 and C-9″ revealed the position of the caffeic acid residue at C-3. Thus, the structure of **2** was assigned as 3-*O*-caffeoyl-2-(2-propyl)-2-hydroxybutanedioic acid, and constitutes an undescribed homologue of **3**.

The HRESIMS of **3** showed a *pseudo*-molecular ion peak [M−H]$^-$ at *m*/*z* 367.1034, consistent with the molecular formula $C_{17}H_{20}O_9$ (calculated for $C_{17}H_{19}O_9$, 367.1029). The 1D- and 2D-NMR data of **3** agreed with that of the previously described, but stereochemically unassigned, 3-*O*-caffeoyl-2-(2-butyl)-2-hydroxybutanedioic acid.[9]

After the planar structures of compounds **1**–**3** were firmly established, the next step was to determine their absolute stereochemistry by the comparison of experimental and calculated IR/VCD data, at the density functional theory (DFT) level. Based on the *erythro* relative configuration assigned for **1** after the translactonization procedure, the only two possible absolute configurations were (2*S*,3*S*) or (2*R*,3*R*). For the calculations of theoretical spectra, the configuration (2*S*,3*S*)-**1** was arbitrarily chosen. In line with results previously obtained for ellagitannins in methanol-d_4[6] the vibrational spectral properties of compound **1** were initially calculated for a deuterated molecule (exchangeable hydrogens attached to oxygen atoms replaced with deuterium). The comparison of experimental IR/VCD data with that calculated for deuterated (2*S*,3*S*)-**1** revealed a nearly mirror image for the two spectra (except for the carbonyl region), thus initially suggesting the absolute configuration of **1** to be (2*R*,3*R*) (Fig. 3). In order to confirm these findings, the same calculations were performed for non-deuterated (2*S*,3*S*)-**1**. Interestingly, the agreement between the experimental and calculated spectra was significantly improved indicating **1** to have the (2*S*,3*S*) absolute configuration. These conflicting results led us to turn our attention to the experimental and calculated IR spectra. The IR band around 1390 cm^{-1} present in the experimental spectrum was correctly reproduced only in the spectra calculated for the non-deuterated molecule (Fig. 3). This broad band arises from O—H bending vibrations of the hydroxyl groups bonded to C-2 and C-3 mostly coupled to C—H, CH_2 (wagging) and CH_3 (symmetric) bendings. Upon deuteration, the hydroxyl bending modes were shifted to the region around 950–1000 cm^{-1}. Regarding the VCD spectra, one of the most prominent features, the negative band around 1300 cm^{-1} also presents significant contributions of vibrational modes coupled to the above-mentioned O—H bendings. The uncoupled vibrations resulting from deuteration of the hydroxyl groups gave rise to spectral patterns mostly opposite to that of the experimental spectrum. The combined analysis of IR and VCD properties for deuterated and non-deuterated molecules allowed us to assign the

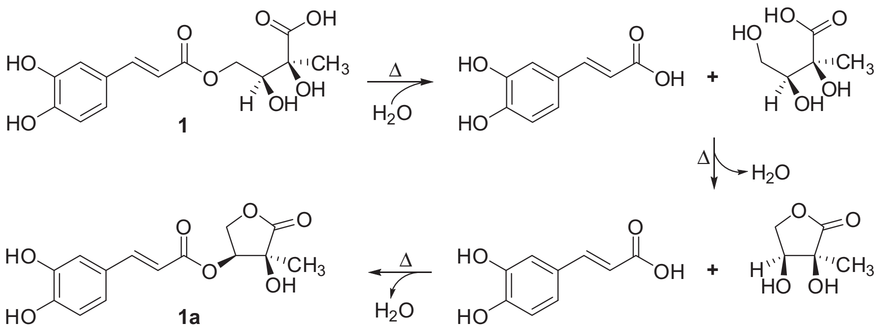

Figure 2. Translactonization of **1** to obtain **1a**.