Abstract
Although tremetone [5-acetyl-2-(1-methylvinyl)-2,3-dihydrobenzofuran] has only one stereogenic center, the absolute configuration (AC) determination of its naturally occurring 11-acyloxy derivatives 1 and 2 by vibrational circular dichroism (VCD) turned out to be difficult. Similarity-based comparison of the experimental VCD spectrum of 11-coumaryloxytremetone (1), isolated from Parastrephia quadrangularis, with spectra calculated using popular density functional theory (DFT) levels of theory, provided poor enantiomeric similarity indices (ESIs), even when the p-coumaroyl ester group of 1 was replaced by the acetyl group in 2. In search for a better understanding of these results, IR-guided individual scaling factors (ISFs), recently introduced as part of the Vibrational Spectra Similarity and Analysis Tool (VISSAT) software, were used to correct DFT frequencies, while a VCD-guided conformational analysis was developed to explore conformational preferences. These studies showed that for both molecules 72% of the individual conformations gave ESI values in favor of the (R) enantiomer. Likewise, when conformer abundances were optimized to produce the best possible similarity for each enantiomer, the obtained ESI values were always larger for the (R) isomer than for the (S) isomer. These results point toward the (R) AC in both compounds and highlight the incorrect conformer abundance prediction by DFT calculations as the potential source of the initial difficulties. In addition, the AC of 1 was independently verified using the Flack and Hooft parameters gained after a single-crystal x-ray diffraction (XRD) study.

Keywords
absolute configuration, Parastrephia species, vibrational circular dichroism, single-crystal x-ray diffraction, 11-coumaryloxytremetone, 11-acetyloxytremetone

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Introduction
Levorotatory 11-coumaryloxytremetone (1) (Figure 1) was first isolated1 from the Bolivian roots of Parastrephia lepidophylla (Wedd.) Cabr. (Asteraceae) and its structure established from spectroscopic data, mainly 1H NMR measurements. It was later isolated2 from the aerial parts of Chilean Parastrephia quadrangularis (Meyen) Cabr. In an independent contemporary study,3 a levorotatory sample of (-)-11-acyloxytremetone (2) was associated to the (R) absolute configuration (AC) by chemical correlation. In addition, naturally occurring benzodihydrofurans 1 and 2 were successfully tested4 as growth inhibitors of the insect species Tenebrio molitor, while the structure of 1 was independently verified5 in a single-crystal x-ray diffraction (XRD) study.6

From our three lustrous vibrational circular dichroism (VCD) experience, accumulated since the first study6 of alkaloids from plants of Chilean origin, through the years during which a few VCD reviews7,8 were put forward,9 until current times,10 it could be expected that the structural attributes of 1 would allow a straightforward AC determination of the molecule. Inspection of the structure of 1 reveals that this C22H20O5 compound possesses only 4 sp3 carbon atoms of which one is a methyl group, a sole stereogenic center which is located at C-2 on a benzodihydrofuran ring system and has an almost rigid p-hydroxycinnamoyl ester group whose phenolic...
hydroxy group can adopt 2 orientations. However, attempts to determine the AC of 1 by commonly used VCD procedures failed. To our surprise, routine computer modeling of 1 using widely used software, as will be detailed in the next section, provided an unexpected very high number of conformers which might be the source of the difficulties.

Moreover, other naturally occurring compounds with a high conformational flexibility have also shown unexpected problems in their VCD studies, apparently related to their complex conformational distributions. This situation has encouraged us to develop new computational tools that could aid in cases were widely used VCD methodologies fail. This effort rendered in the creation of a software named Vibrational Spectra Similarity and Analysis Tool (VISSAT), a group of computational tools that allows to conduct similarity calculations between observed and calculated IR/VCD spectra, as well as the search and optimization of different spectra parameters of interest. In the present study, we used VISSAT to conduct a very detailed stereochemical and conformational analysis of 1 and 2 to shed some light on the underlying causes that prevent obtaining satisfactory results when traditional VCD methodologies are used.

In the present, predominantly computational work, some limitations of density functional theory (DFT) VCD calculations for AC assignments are highlighted. Considering that AC determinations by VCD are relevant mainly for pharmaceutical industry, where in many cases band analogy is invoked, and for natural products where unexpected molecules can still be found, the present forum for the publication of these results seems ideal.

Results and Discussion

A molecular model of (R)-11-coumaryloxytremetone (1) was constructed in the Spartan 14 software (Wavefunction, Inc.) and a conformational search, using the Monte Carlo protocol and MMFF94 molecular mechanics force field, was performed using the same software. To our surprise, this provided a huge amount of 278 conformers in the lowest 10 kcal/mol energy gap. The surprise derives from the fact that the molecule has 22 carbon atoms of which only 4, including a methyl group, are sp³ hybridized. DFT geometry optimizations performed at the B3LYP/DGDZVP2 level of theory reduced this set to 183 conformers after the removal of duplicated structures since several models converged into the same conformer. From the optimized molecular geometries, frequency calculations at the same level of theory afforded dipole and rotational strengths, along with Gibbs free energies for each conformation, showing that the 87 conformers found in the initial 2 kcal/mol energy gap accounted for 93.5% of the entire Boltzmann conformational distribution. This very large set of conformers was used to generate weighted IR and VCD spectra for 1 (Figure 2, left).

Calculated frequencies, gained from the same calculations, were scaled with an anharmonicity or global scale factor (GSF) of 0.984, found as the optimal value for the maximization of the IR similarity index function (SIR),¹ which in turn showed only a modest matching value of around 0.85 (Table 1). Furthermore, comparison between calculated and observed VCD spectra using the enantiomeric similarity index (ESI)¹³, which is the VCD similarity difference between the calculated isomer (S1) and its enantiomer (S10), showed a close to zero ESI value. The inclusion of conformers lying above the initial 2 kcal/mol limit into the conformational distribution produced nearly identical IR/VCD spectra and showed no improvement in the SIR and ESI values. These results suggest that the AC of 1 cannot be determined by VCD, albeit such a task looked simple at first glance.

In an attempt to overcome this adverse situation, the molecular size of the studied tremetone derivative was reduced by chemical means, thereby reducing the molecular flexibility. Thus, the replacement of the p-hydroxycumaryl ester residue of 1 for an acetyl group in 2, by hydrolysis followed by acetylation, allowed a significant simplification of the conformational picture, from 278 to 58 conformers, in the initial Monte Carlo conformational searches, and from 183 to 50 unique conformers after DFT geometry optimizations. This change is not affecting the sole stereogenic center and speeded up the calculation procedures since 1 possesses 192 electrons to be considered in the DFT calculations, while 2 possesses only 138 electrons.

As in the case of 1, weighted IR and VCD spectra for 2 were generated and compared with the corresponding observed traces (Figure 2, right) using similarity functions SIR and ESI. The SIR value showed a slight improvement to 0.876 (Table 1), while the VCD spectra matching improved to an ESI value of 0.322, thereby showing a clear preference for the (R)-2 enantiomer.

In attempts to improve these results, several combinations of DFT functionals and basis sets were tested for 2. The

![Figure 1. Formulas of 11-acyloxytremetones.](image-url)
results, summarized in Table 2, show that similarity values did not improve throughout the tested levels of theory, with \( \text{GSF} \)-\( S_{\text{IR}} \) values in the 0.81 to 0.88 range and low \( \text{GSF} \)-\( S_{\text{ESI}} \) values, although in general pointing toward the (\( R \)) enantiomer, as occurred with the first B3LYP/DGDZVP2 tested level of theory.

At this point, a search was started to find procedures that might improve the matching between theory and observation, and eventually explain the reasons why a widely used and successful methodology, like VCD, was not providing adequate results in cases like those studied herein.

First, the nonoptimal obtained \( S_{\text{IR}} \) values suggest that calculated frequencies are not satisfactorily matching the experimental data. Since it was recently evidenced\(^{11} \) that instead of using an anharmonicity (\( \text{anH} \)) factor\(^{13} \) which means a global scaling factor (\( \text{GSF} \)), calculated frequencies can be scaled more efficiently using individual scaling factors (\( \text{ISF} \)s) to generate an optimized \( S_{\text{IR}} \) value, and eventually improve \( E_{\text{SI}} \) values. To obtain these \( \text{ISF} \)s values, a sequential search algorithm was applied to the most abundant conformer, which is then used for all remaining conformers, thus yielding very high \( S_{\text{IR}} \) values. This computational procedure, included in the recently created VISSAT software\(^{11} \), showed to be effective for the probe molecule 3-methylcyclopentanone\(^{11} \), but gave unsatisfactory results when it was applied to compound 2, most likely due to the much larger number of vibrational modes and conformations found in 2. Therefore, improvements in the \( \text{ISF} \)s search algorithm of VISSAT were needed to successfully use the technique for this much more flexible compound. To start with, the sequential search algorithm was replaced by an iterative minimization algorithm which searches for an optimal set of \( \text{ISF} \)s

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**Figure 2.** Comparison of observed and calculated IR (lower traces) and vibrational circular dichroism (VCD) (upper traces) spectra of 1 (left) and 2 (right) at the B3LYP/DGDZVP2 level of theory. Global scale factor (\( \text{GSF} \)) of 0.984 and 0.970 were used for 1 and 2, respectively.

**Table 1.** Similarity values\(^a \) of Observed and Calculated IR (\( S_{\text{IR}} \)) and vibrational circular dichroism (VCD) (\( S_{\text{IR}}-S_{\text{E}} = E_{\text{SI}} \)) spectra of 1 and 2 Using the B3LYP/DGDZVP2 Level of Theory.

| Index | 2 kcal | Total | 2 kcal | Total |
|-------|--------|-------|--------|-------|
| \( S_{\text{IR}} \) | 0.843 | 0.845 | 0.876 | 0.876 |
| \( S_{\text{E}} \) | 0.215 | 0.226 | 0.455 | 0.455 |
| \( S_{\text{E}} \) | 0.237 | 0.228 | 0.133 | 0.133 |
| \( E_{\text{SI}} \) | -0.022 | -0.001 | 0.322 | 0.322 |

\(^a \)\( S_{\text{IR}} \): IR spectral similarity; \( S_{\text{E}} \): VCD spectral similarity for the (\( R \)) enantiomer; \( S_{\text{E}} \): VCD spectral similarity for the (\( S \)) enantiomer; \( E_{\text{SI}} \): enantiomeric similarity index (\( S_{\text{E}}-S_{\text{E}} \)).
Table 2. Similarity values of Observed and Calculated IR (SIR) and vibrational circular dichroism (VCD) (SIR−SIR = ESI) spectra of 2 at Different Levels of Theory and Frequency Scaling Procedures.

| Functional | Basis set | SIR | ISF | SIR | ISF | SIR−E | ISF | ESI |
|------------|-----------|-----|-----|-----|-----|-------|-----|-----|
| B3LYP      | DGDZVP    | 0.840 | 0.974 | 0.430 | 0.481 | 0.140 | 0.126 | 0.230 | 0.355 |
|            | DGDZVP2   | 0.876 | 0.987 | 0.455 | 0.473 | 0.133 | 0.095 | 0.322 | 0.378 |
|            | cc-pVDZ   | 0.857 | 0.978 | 0.210 | 0.388 | 0.277 | 0.168 | −0.067 | 0.219 |
| B3PW91     | DGDZVP    | 0.836 | 0.980 | 0.351 | 0.399 | 0.139 | 0.126 | 0.211 | 0.273 |
|            | DGDZVP2   | 0.835 | 0.980 | 0.345 | 0.393 | 0.140 | 0.128 | 0.205 | 0.265 |
|            | cc-pVDZ   | 0.821 | 0.960 | 0.200 | 0.342 | 0.269 | 0.168 | −0.069 | 0.174 |
| PBEPBE     | DGDZVP    | 0.826 | 0.946 | 0.514 | 0.485 | 0.162 | 0.131 | 0.352 | 0.354 |
|            | DGDZVP2   | 0.835 | 0.975 | 0.424 | 0.413 | 0.142 | 0.141 | 0.282 | 0.272 |
|            | cc-pVDZ   | 0.815 | 0.964 | 0.413 | 0.393 | 0.108 | 0.189 | 0.305 | 0.204 |

*SIR: IR spectral similarity; SIR: VCD spectral similarity for the (R) enantiomer; SIR−E: VCD spectral similarity for the (S) enantiomer; ESI: enantiomeric similarity index (SIR−SIR).

Table 3. Wave Numbers (cm⁻¹) of the Vibrational Modes Using Different Scaling Procedures and Their Corresponding Scale Factors for the Global Energy minimum Conformation of 2 at the B3LYP/DGDZVP2 Level of Theory.

| Mode | Wave numbers | Scale factors |
|------|--------------|---------------|
|      | DFTV | GSFV | ISFV | GSF | ISF | GSF | ISF |
| 54   | 1136.6 | 1102.4 | 1117.7 | 0.970 | 0.980 | 0.983 |
| 55   | 1179.1 | 1143.6 | 1158.0 | 0.980 | 0.982 |
| 56   | 1186.5 | 1150.8 | 1164.6 | 0.980 | 0.982 |
| 57   | 1240.5 | 1203.1 | 1218.0 | 0.980 | 0.982 |
| 58   | 1254.8 | 1217.0 | 1225.7 | 0.980 | 0.977 |
| 59   | 1260.2 | 1222.2 | 1234.4 | 0.980 | 0.980 |
| 60   | 1274.3 | 1235.9 | 1247.9 | 0.980 | 0.979 |
| 61   | 1294.0 | 1255.0 | 1268.5 | 0.980 | 0.980 |
| 62   | 1306.6 | 1267.2 | 1276.6 | 0.980 | 0.977 |
| 63   | 1328.8 | 1288.8 | 1305.9 | 0.980 | 0.983 |
| 64   | 1340.7 | 1300.3 | 1316.6 | 0.980 | 0.982 |
| 65   | 1353.6 | 1312.8 | 1321.9 | 0.980 | 0.977 |
| 66   | 1377.2 | 1335.7 | 1353.9 | 0.980 | 0.983 |
| 67   | 1383.4 | 1341.7 | 1358.5 | 0.980 | 0.980 |
| 68   | 1385.6 | 1343.9 | 1356.2 | 0.980 | 0.979 |
| 69   | 1403.9 | 1361.6 | 1370.6 | 0.980 | 0.976 |
| 70   | 1462.8 | 1418.7 | 1431.5 | 0.980 | 0.979 |
| 71   | 1470.9 | 1426.6 | 1438.1 | 0.980 | 0.978 |
| 72   | 1471.5 | 1427.1 | 1438.1 | 0.980 | 0.977 |
| 73   | 1472.8 | 1428.5 | 1438.4 | 0.980 | 0.977 |
| 74   | 1477.8 | 1433.3 | 1443.6 | 0.980 | 0.977 |
| 75   | 1482.0 | 1437.3 | 1446.5 | 0.980 | 0.976 |
| 76   | 1483.9 | 1439.2 | 1450.3 | 0.980 | 0.977 |
| 77   | 1491.7 | 1446.8 | 1459.0 | 0.980 | 0.978 |
| 78   | 1525.8 | 1479.8 | 1488.7 | 0.980 | 0.976 |
| 79   | 1636.5 | 1587.2 | 1593.1 | 0.970 | 0.973 |
| 80   | 1653.1 | 1603.3 | 1604.1 | 0.970 | 0.970 |
| 81   | 1716.6 | 1664.9 | 1652.7 | 0.960 | 0.963 |
| 82   | 1736.9 | 1684.5 | 1669.2 | 0.960 | 0.961 |
| 83   | 1799.0 | 1744.8 | 1738.6 | 0.970 | 0.966 |

*DFTV: density functional theory value; GSFV: global scale factor value; ISFV: individual scale factor value.

*GSF: global scale factor; ISF: individual scale factor; SSP, search starting point.
VCD spectra, with $S_{IR}$ and ESI similarity values of 0.987 and 0.309, respectively.

Considering the above result, it was decided to engage in a deeper analysis of the conformational preferences of 2, aiming to overcome the difficulties shown by DFT calculations to correctly predict the conformational distribution. As a first approach, $S_{IR}$ and ESI values were collected individually for each conformer, searching for models that may be underestimated by the DFT calculations. As shown in Figure 3, the values of $S_{IR}$ remain mostly unchanged with values slightly below the weighted value. In addition, 72% of the conformers show positive ESI values, several of them larger than the weighted VCD spectrum, and many with very small abundances assigned by the DFT calculations. These results suggest that a different conformational distribution, in which these conformations have larger abundances, should produce a different weighted VCD spectrum with a better agreement with the observed VCD plot.

From the mathematical standpoint of view, a weighted spectrum is a linear combination of the individual spectra produced by all possible conformers of a given compound, in which the contributions of each conformation depend on their relative stability. If these individual spectra are being correctly predicted by DFT calculations, and frequency shifts are being corrected to a high degree, it follows that spectra similarity will mostly depend on these individual contributions, which are commonly obtained from the same ab initio calculations. Nevertheless, it has been shown that DFT calculated thermochemical parameters can show significant errors, even when high-end levels of theory and very dense integral grids are used. Such errors appear to be particularly troublesome in highly flexible compounds, were the appearance of low-frequency vibrational modes (5-50 cm$^{-1}$) produce severe errors in the computed entropy contributions to the Gibbs free energies. These low energy modes, that can be characterized as internal rotations of functional groups, rather than as molecular vibrations, could be the main source of the difficulties found herein and in other cases.

Alternatively, applying the same principle used by VISSAT to obtain a set of IF values that maximizes IR similarity, it is also possible to search for the optimal linear combination that maximizes similarity between observed and calculated VCD spectra. Since enantiomers produce antipode VCD spectra, the searches need to be performed independently for each enantiomer, producing separated similarity values for the (R) and the (S) enantiomer. With this idea in mind, an iterative optimization algorithm that uses the DFT relative abundances as starting points was implemented in the VISSAT software to search for new conformational distribution sets that maximize VCD similarity. These procedures were performed using the IF scaled frequencies, with their corresponding dipole and rotational strengths, calculated at the B3LYP/DGDZVP2 level of theory for each conformer of 2. This provided two new conformational distribution sets, one derived from optimizing against each enantiomer [(R)-guided and (S)-guided]. Using these optimized abundances, large similarity increments were obtained between the resulting weighted and observed VCD spectra, in comparison to those obtained from DFT free energy calculations. When the VCD spectrum calculated for the (R) enantiomer was used, the new VCD-guided conformational distribution produced a very large $S_{E}$ value of 0.890, while a considerable smaller $S_{E}$ value of 0.572 was obtained when the VCD spectrum of the (S) enantiomer was used to optimize the conformational distribution. Moreover, these VCD similarity increments translated into ESI value improvements, which in the case of the (R) enantiomer conformational distribution advanced to 0.825, while for the (S) enantiomer it provided −0.493. It is important to notice that the negative sign of the later ESI value indicates a preference for the other enantiomer.

At first glance, these results seem to suggest that the entire conformational set is needed to produce a weighted VCD spectrum having high similarity with the observed spectrum. However, the computational algorithm will use the best available spectra combination from a given set, even when it is possible that other combinations, composed by more stable conformers, as determined by DFT free energies, could also have high similarities. To test this, VCD-guided conformational distributions, using progressively smaller conformer sets were obtained by decreasing the energy threshold of each distribution and calculating the corresponding similarity values. As shown in Figure 4, several interesting features appear when ESI values are correlated with the considered conformational energy window. First, the contraction of the conformational pool tends to have a detrimental effect over the final similarity obtained for each conformational distribution, and remains high with a small decrease below 1.5 kcal/mol. This is in line with previous observations made in the VCD study of catechin and epicatechin peracetates, where consideration of conformers above this energy threshold appears to be unnecessary to allow a good prediction of the conformational distribution. Furthermore, the preference toward the (R) enantiomer is a constant throughout the energy window, pointing toward this as the correct AC.

When only the most stable conformational distributions are constructed using restricted energy windows, like those on the left-hand side of the graph, positive or very small negative ESI values are obtained for the $S$-guided distributions, while ESI values above 0.5 are observed for the R-guided counterparts. Since these conformations correspond to the most stable conformers, this suggests that these calculations are roughly predicting the group of most abundant conformations, although not accurately enough to properly predict their abundances.

The general analysis used for the smaller acetate 2 was then applied to the $p$-coumarate 1 calculated at the B3LYP/DGDZVP2 level of theory. This yielded the results shown in Figures 5 and 6. As with 2, most conformations (72%) show positive ESI values and the preference for the (R) enantiomer is observed when the full conformational set is considered, with ESI values for the (R)-guided and the (S)-guided spectra of 0.881 and −0.776, respectively. Unlike 2, in this case, ESI differences between both conformational distributions become less clear at lower energy conformations, suggesting a much more complex scenario for the larger compound. Nevertheless, for
the lowest energy conformations, very small negative ESI values are observed again for the \(S\)-guided distributions in opposition to the \(R\)-guided distributions, for which values around 0.5 are obtained.

As shown in Figure 7, a VCD-guided conformational distribution produced highly similar IR and VCD spectra when compared with the corresponding observed spectra, that in turn contrast with their free energy counterparts (Figure 2), clearly suggesting the incapacity of the DFT calculations to predict correct conformational abundances.

It is of relevance to note that the VCD spectra of 1 and 2 (Figure 2) show mostly negative bands, which at first glance

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**Figure 3.** Abundances (upper trace), IR similarities (\(S_{IR}\)) (central trace), and vibrational circular dichroism (VCD) enantiomeric selectivity indexes (ESI) (bottom trace) of each conformation of 2 calculated at the B3LYP/DGDZVP2 level of theory. Individual scaling factors (ISFs) obtained for the IR weighted spectra were used in all cases.
is surprising. This behavior might be attributed to instrumental artifacts, measurement deficiency, or to the sample itself. Consequently, the spectra were carefully recorded several times over different days using correct operation conditions and the identity and purity of the samples were always checked by H-NMR measurements, so the mainly mono-signated spectra shapes seem to own the sample. In fact, the VCD spectra of \( \text{1} \) and \( \text{2} \) remember the spectra of \( 7\text{-O-}\beta\text{-D-glucopyranosylchrysin, 21 of (}S\text{-})\text{-5-hydroxy-7-methoxy-4'}\text{-acetyloxyflavanone, 22 and of podocephalol acetate. 23 For the later compound supramolecular studies could be done.} \)

As already mentioned in the introduction, some limitations of DFT VCD calculations for AC assignments are highlighted. It is just of further relevance to mention that they are in line with other natural products cases like perezone\(^{17,18} \) and guaiaretic acid diacetate.\(^{19} \)

Although a single-crystal XRD study of 11-coumaryloxytremetone (\( \text{1} \)) has already been reported,\(^{3} \) which revealed that the molecule provides orthorhombic \( P2_12_12_1 \) non-centro-symmetric crystals, the AC was not verified at that time. Thus, to independently determine the tridimensional structure of the molecule, another XRD study was undertaken since the AC was not tested in the original XRD study. This allowed to ascertain the (R) AC of \( \text{1} \) from the Flack parameter,\(^{24} \) which was \( x = -0.2(3) \), and the Hooft parameter,\(^{25,26} \) which was \( y = -0.16(17) \). These parameters were \( x = 1.1(3) \) and \( y = 1.15(17) \) for the inverted structure. A PLUTO plot of the XRD structure is shown in Figure 8.

Once some limitations of classical DFT VCD calculations to determine the AC of \( \text{1} \) and \( \text{2} \) by VCD were overcome, there are some general considerations about the used methodology that might be of interest to comment. Our approach to initially correct anharmonicity on each conformer and then to estimate conformers contributions seems for the moment a plausible strategy. Before the advent of the Compare\(^{1/0}A \) software, an anharmonicity factor of 0.97 was generally applied to the calculated frequencies. Afterward, the methodology of Stephens\(^{27-29} \) was applied. This is a manual band-to-band plot comparison of calculated and experimental frequencies. A line with positive slope indicated the correct AC and the root-mean-squares error calculations indicated the quality of the fit. In certain sense, this is indicative of the ISF that would be required for a perfect fit. This methodology was used for guaiaretic acid diacetate\(^{19} \) after the failure of classic approaches.

**Conclusions**

Albeit the AC determination of 11-coumaryloxytremetone (\( \text{1} \)) by VCD looked like a straightforward task, the procedure turned out to be quite complex. Commonly used DFT calculated spectra comparison with an experimental spectrum was useless. The replacement of the \( p\)-coumaroyl group for an acetyl grout speeded up the calculation process due to the removal of 54 electrons from the molecule and a substantial decrease in the number of conformers to be considered, but only produced a modest increase in similarity values. The (R) AC configuration of the studied 11-acyloxytremetones \( \text{1} \) and \( \text{2} \) could confidently be determined herein using the very recently developed VISSAT, which allowed a conformationally resolved analysis of the similarity between DFT calculated and observed VCD spectra. These analyses suggest that DFT conformational distributions appear to be weak links in the process of predicting the VCD spectra of highly flexible molecules such
as those studied herein. The studies can also tip the balance when a clear preference for a particular enantiomer is encountered, but somehow similarity values are not satisfactory to obtain a reliable AC assignment through classical comparison procedures. Such methodologies, like the widely used CompareVOA software, remains as an extremely useful strategy for the correct identification of the AC of many natural products. However, it seems there is a small category of molecules that we could classify as “VCD rebel natural products” which have to be evaluated using an alternative methodology like the one outlined herein. The AC determination of tremetone derivatives 1

Figure 5. Abundances (upper trace), IR similarities ($S_{IR}$) (central trace), and vibrational circular dichroism (VCD) enantiomeric selectivity indexes ($ESI$) (bottom trace) of each conformation of 1 calculated at the B3LYP/DGDZVP2 level of theory. Individual scaling factors ($ISF$) obtained for the IR weighted spectra were used in all cases.
Figure 6. Vibrational circular dichroism (VCD) enantiomeric selectivity indexes (ESI) of 1 at different energy windows from R-guided (squares), S-guided (dots), and density functional theory (DFT) (triangles) abundances.

Figure 7. Comparison of observed and calculated IR (lower traces) and vibrational circular dichroism (VCD) (upper traces) spectra of 1 (left) and 2 (right) at the B3LYP/DGDZVP2 level of theory using VCD-guided abundances.
and 2 is, by far, the most difficult VCD task we ever faced for molecules possessing a single stereogenic center.

**Experimental Section**

11-Acyloxytremetones

Pure 11-coumaryloxytremetone (1) was available from a previous study, while 11-acetyloxytremetone (2), which is also a natural product, was prepared by simple alkaline hydrolysis of 1 followed by routine acetylation. The identity and purity of both compounds were verified by 300 MHz 1H-NMR measurements in CDCl₃ solutions, containing TMS as the internal reference, on a Varian Mercury 300 NMR spectrometer.

VCD Measurements

Samples of 6.5 mg of 1 and of 5.3 mg of 2, dissolved in 150 μL of 100% atom-D CDCl₃, were placed in cells equipped with BaF₂ windows and a path length of 0.1 mm. This provided the most intense IR band absorptions in the A = 0.7 to 0.8 range. Data were acquired on a BioTools dualPEM ChiralIR FT spectrophotometer at a resolution of 4 cm⁻¹ averaging 61 h blocks. The baseline was corrected by subtracting the spectrum of the solvent which was acquired under identical instrumental conditions. The stability of the studied molecules was monitored by 300 MHz 1H NMR measurements performed immediately before and after the VCD determinations.

Vibrational Circular Dichroism Calculations

Monte Carlo conformational searches for 1 and 2 were performed using the Spartan 14 software suite (Wavefunction, Inc.), while all the remaining DFT calculations were performed using the Gaussian 03W software (Gaussian Inc.). Geometry optimizations followed by frequency analysis were performed for each conformation of both compounds using the tight convergence option, while the UltraFine (150,974) DFT integral grid was selected in all calculations. The coding and subsequent similarity calculations were performed using MATLAB R2020b (The MathWorks Inc.), and IR/VCD spectra were plotted as Lorentzian bands with half-widths of 8 cm⁻¹. Molecular visualization was carried out with the GaussView 5.0 program. Geometry optimizations and vibrational calculations were performed using an HP ProLiant DL320e Server with an Intel Xeon E3-1220 CPU operating at 3.1 GHz with 8 GB RAM, while data processing was performed in a PC with an Intel Core i7-10700 CPU operating at 2.9 GHz with 32 GB RAM.

Single-Crystal x-ray Diffraction Study of 11-Coumaryloxytremetone (1)

Suitable colorless crystals of (1) were grown by slow evaporation from an ethyl acetate/hexanes solution. A crystal measuring 0.16 × 0.12 × 0.08 mm was trapped in mineral oil on a PTFE loop and data were collected on a Bruker D8 Venture diffractometer equipped with graphite monochromated Cu Kα (λ = 1.54184 Å) radiation in the θθθ and θωω scan modes. Unit cell refinements using 9531 machine detected reflections were done with the APEX3 v2016.1 (Bruker AXS Inc., 2016) software. The crystal was orthorhombic, space group P2₁2₁2₁, with cell dimensions a = 6.2113(3) Å, b = 13.1735(7) Å, c = 22.8332(7) Å, V = 1868.3(2) Å³, ρcalc = 1.295 g/cm³ for Z = 4, C₂₂H₂₀O₅, MW = 364.38, μ = 0.752 mm⁻¹, T = 298(2) K, and F(000) = 768 e. A total of 43,448 reflections were collected, of which 3971 unique reflections (Rint = 0.1139), with I > 4σ(I) were used for the study. The structure was solved by direct methods using the SIR2004 software and refined by full-matrix

![Figure 8. PLUTO plot of the x-ray crystal structure of 11-coumaryloxytremetone (1).](image-url)
least squares on F2. The non-hydrogen atoms were refined anisotropically and the hydrogen atoms were added at geometrically idealized positions on their parent atoms. The observed reflections were 2996, and final discrepancy indices, refining 258 parameters, were $R_p = 5.3\%$ and $R_w = 14.6\%$. Goodness-of-fit on $R^2 = 1.042$. The final difference Fourier map was essentially featureless, the highest residual peak and hole having residual densities of 0.167 and $-0.136 \text{ e/Å}^3$, respectively. For the AC determination, the Flack parameter $x = -0.2(3)$ and Hooft parameter $y = -0.16(17)$ were used, which for the inverted structure were $x = 1.1(3)$ and $y = 1.15(17)$. The PLUTO plot of XRD structure is shown in Figure 8. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre, under deposition number 2141969, from where they can be obtained, free of charge, via http://www.ccdc.ac.uk/data_request/cif.

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Declaration of Conflicting Interests

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Ethical Approval

Ethical approval is not applicable for this article.

Statement of Human and Animal Rights

This article does not contain any study with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Trial Registration

Not applicable, because this article does not contain any clinical trials.

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References

1. Bohlmann F, Fritz U, King RM. Neue tremeton-derivate aus Parastrephia lepidophylla. Phytochemistry. 1979;18(8):1403-1405. https://doi.org/10.1016/0031-9422(79)83037-X
2. Loyola LA, Naranjo J, Morales G. 5,7-Dihydroxy-3,8,3’,4’-tetramethoxyflavone From Parastrephia quadrangularis. Phytochemistry. 1985;24(8):1871-1872. https://doi.org/10.1016/S0031-9422(85)82580-7
3. Kawase Y, Yamaguchi S, Inoue O, Sanomilla M, Kawabe K. The synthesis and absolute configuration of fommannosin, (−)-5-acetyl-2-(1-hydroxymethylvinyl)-2,3-dihydrobenzofuran, and anodendroic acid. Chem Lett. 1980;9(12):1581-1584. https://doi.org/10.1246/cl.1980.1581
4. Carrizo R, Sosa ME, Favier LS, et al. Growth-inhibitory activities of benzofuran and chromene derivatives toward Tenebrio molitor. J Nat Prod. 1998;61(10):1209-1211. https://doi.org/10.1021/np9800248
5. Brito I, Simirgiotis M, Muñoz R, et al. Crystal structure of 11-coumaryloxytremetone, $C_{24}H_{20}O_4$. Z Kristallogr NCS. 2017;232(1):13-14. https://doi.org/10.1515/nzcrs-2016-0105
6. Muñoz MA, Muñoz O, Joseph-Nathan P. Absolute configuration of natural diasteroisoomers of $\beta$-hydroxyhyoscyamine by vibrational circular dichroism. J Nat Prod. 2006;69(9):1335-1340. https://doi.org/10.1021/np060133j
7. Joseph-Nathan P, Gordillo-Román B. Vibrational circular dichroism absolute configuration determination of natural products. In: Kinghorn AD, Falk H, Kobayashi J, eds. Progress in the Chemistry of Organic Natural Products. Springer International Publishing; 2015;100:311-452. https://doi.org/10.1007/978-3-319-05275-5_4
8. Burgueño-Tapia E, Joseph-Nathan P. Vibrational circular dichroism: recent advances for the assignment of the absolute configuration of natural products. Nat Prod Commun. 2015;10(10):1785-1795. https://doi.org/10.1177/21934578X1501001036
9. del Río RE, Joseph-Nathan P. Vibrational circular dichroism absolute configuration of natural products from 2015 to 2019. Nat Prod Commun. 2021;16(3):1-30. https://doi.org/10.1177/21934578X21996166
10. Fuentes-Figueiroa MA, Joseph-Nathan P, Burgueño-Tapia E. Absolute configuration assignment of stigmasterol oxiranes. Chirality. 2022;34(2):396-420. https://doi.org/10.1002/chir.23390
11. Muñoz MA, Joseph-Nathan P. Vibrational circular dichroism enantiomeric similarity index improvement for absolute configuration discrimination using individual infrared scaling factors. Chirality. 2022;34(3):559-570. https://doi.org/10.1002/chir.23411
12. Burgueño-Tapia E, Zepeda LG, Joseph-Nathan P. Absolute configuration of (−)-myrtenal by vibrational circular dichroism. Chiroptemistry. 2010;71(10):1158-1161. https://doi.org/10.1016/j.chircom.2010.04.005
13. Debie E, Gussem ED, Dukor RK, Herrebout W, Naïf LA, Bulink P. A confidence level algorithm for the determination of absolute configuration using vibrational circular dichroism or Raman optical activity. Chem Phys Chem. 2011;12(8):1542-1549. https://doi.org/10.1002/cphc.201100050
14. Kruse H, Goerigk I, Grimme S. Why the standard B3LYP/6-31G* model chemistry should not be used in DFT calculations of molecular thermochemistry: understanding and correcting the problem. *J Org Chem.* 2012;77(23):10824-10834. https://doi.org/10.1021/jo302156p

15. Martin JML, Bauschlicher CW, Ricca A. On the integration accuracy in molecular density functional theory calculations using Gaussian basis sets. *Comput Phys Commun.* 2001;133(2-3):189-201. https://doi.org/10.1016/S0010-4655(00)00174-0

16. Pracht P, Grimme S. Calculation of absolute molecular entropies and heat capacities made simple. *Chem Sci.* 2021;12(19):6551-6568. https://doi.org/10.1039/D1SC00621E

17. Rojo-Portillo T, Reyes-López E, Hernández-Huerta E, et al. Is the VCD spectrum a fingerprint of the conformational population? The conformation of perezone in the spotlight. *J Mol Struct.* 2020;1202:127273. https://doi.org/10.1016/j.molstruc.2019.127273

18. Burgueño-Tapia E, Cerda-García-Rojas CM, Joseph-Nathan P. Conformational analysis of perezone and dihydroperzones using vibrational circular dichroism. *Phytochemistry.* 2012;74:190-195. https://doi.org/10.1016/j.phytochem.2011.10.005

19. Ortega AR, Burgueño-Tapia E, Joseph-Nathan P. Difficulties to determine the absolute configuration of guaiaretic acid. *Nat Prod Commun.* 2018;13(8):981-986. https://doi.org/10.1177%2F1934578X1801300815

20. Burgueño-Tapia E, Joseph-Nathan P. Optimization of the number of considered conformers for the absolute configuration determination of catechin and epicatechin peracetates by VCD. *Nat Prod Commun.* 2017;12(5):683-686. https://doi.org/10.1177%2F1934578X1701200510

21. Quevedo-Tinoco I, Rodríguez-García G, del Río RE, et al. Strategy for the vibrational circular dichroism study of a glycosylflavonoid evaluated as its peraceta. The case of bioactive 7-O-b-D-glucopyranosylchrysin. *J Mol Struct.* 2021;1225:1-9. https://doi.org/10.1016/j.molstruc.2020.129147

22. Valkès E, González C, Díaz K, et al. Biological properties and absolute configuration of flavonones from *Calendaria thyrifera* Graham. *Front Pharmacol.* 2020;11:1-11. doi: 10.3389/fphar.2020.01125

23. Rodríguez-García G, Villagómez-Guzmán AK, Talavera-Alemán A, et al. Conformational, configurational, and supramolecular studies of podocephalol acetate from *Lasianthaea aurea*. *Chirality.* 2019;31(11):923-933. https://doi.org/10.1002/chir.23042

24. Parsons S, Flack HD, Wagner T. Use of intensity quotients and differences in absolute structure refinement. *Acta Crystallogr.* 2013;B69(Part3):249-259. https://doi.org/10.1107/S2052521413001014

25. Hooft RWW, Straver LH, Spek AL. Using the t-distribution to improve the absolute structure assignment with likelihood calculations. *J Appl Crystallogr.* 2010;43(Part4):665-668. https://doi.org/10.1107/S0021889810018601

26. Hooft RWW, Straver LH, Spek AL. Determination of absolute structure using Bayesian statistics on Bijvoet differences. *J Appl Crystallogr.* 2008;41(Part1):96-103. https://doi.org/10.1107/S0021889807059870

27. Stephens PJ, Pan JJ, Krohn K. Determination of the absolute configurations of pharmacological natural products via density functional theory calculations of vibrational circular dichroism: the new cytotoxic iridoid prismatomerin. *J Org Chem.* 2007;72(20):7641-7649. https://doi.org/10.1021/jo071183b

28. Stephens PJ, Pan JJ, Devlin FJ, Krohn K, Kurtan T. Determination of the absolute configurations of natural products via density functional theory calculations of vibrational circular dichroism, electronic circular dichroism and optical rotation: the iridoids plumericin and isoplumericin. *J Org Chem.* 2007;72(9):3521-3536. https://doi.org/10.1021/jo070155q

29. Stephens PJ, Pan JJ, Devlin FJ, Urbanova M, Hajicek J. Determination of the absolute configurations of natural products via density functional theory calculations of vibrational circular dichroism, electronic circular dichroism and optical rotation: the schizogynane alkaloid schioxoygune. *J Org Chem.* 2007;72(7):2508-2524. https://doi.org/10.1021/jo062567p