On the Chiroptical Behavior of Conjugated Multichromophoric Compounds of a New Pseudoaromatic Class: Bicolchicides and Biisocolchicides

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Abstract

Background: It is well known that, stemming from the mutual interplay between chromophores, circular dichroism (CD) is a powerful technique to deal with structural problems for both the small organic molecule and the biopolymer. However, quantitative interpretations of the spectroscopic and structural terms that give rise to the exciton couplet are usually presented for ideal cases, or a few CD bands only are taken into account, overlooking the role of the solvent medium.

Methodology/Principal Findings: Circular dichroism and UV absorption spectra were carried out for colchicide (3) and isocolchicide (6), as well as their coupling products, 10,10'-bicolchicide (2) and 9,9'-biisocolchicide (5), in both hydrogen bonding and non hydrogen bonding solvents, as well as MeCN/H2O mixtures. A dramatic control by the solvent emerged, as even tiny changes in the composition of solvent mixtures, at ca 1 water molar fraction, induced a dramatic modification of their CD bands. A mutarotation phenomenon - long known for isocolchicine (8) - was also observed for 5, and can be attributed to the interconversion between atropisomers $(R_a,7S),(R_a,7'S)$-5a and $(R_a,7S),(S_a,7'S)$-5b.

Conclusions/Significance: Our data show that with molecules built on two structurally identical moieties which embody both hydrophilic and hydrophobic groups, even tiny changes in the composition of solvent mixtures cause a dramatic modification of the CD bands. Their analysis arrives at a qualitative rationalization of the observed CD couplets from the coupling of high energy transitions, while attempts at a quantitative interpretation of these phenomena through time-dependent density functional theory allowed to reproduce satisfactorily the CD spectrum in the 300–450 nm region only. Failure with higher energies probably reflects currently inadequate specific theoretical treatments of the solvent medium.

Introduction

Circular dichroism (CD) is a powerful technique to deal with structural problems for both the small organic molecule and the biopolymer. This stems from the mutual interplay between chromophoric units, which affects the molecular properties. Under favorable circumstances, when two or more equivalent chromophores - which absorb light strongly in the same spectral region - are present in a molecular frame at suitable mutual distance and orientation, the CD spectral features (e.g. exciton couplets) may offer a clue as to the stereochemistry of the molecule [1], [2].

Models of such systems, built from either classical electrodynamics [3], [4] or quantum mechanics [5], can also allow a quantitative interpretation of the spectroscopic and structural terms that give rise to the exciton couplet [6]–[8]. However, except in the near-to-ideal cases, success is often limited to a few bands only. In any case, as expected for calculations with electronically excited states, any quantitative interpretation is faced with problems. This is especially true when the conformational behavior of the molecule depends on the nature of the solvent, like for any theoretical treatment of solvent effects.

Here we present a notable example of solvent-dependent circular dichroism involving multichromophoric systems. It concerns 10,10'-bicolchicide (2) and 9,9'-biisocolchicide (5), where two identical colchicine (1) or isocolchicine (4) chromophores of known absolute configuration $(R_a,7S)$, are linked through a formally single covalent bond (Figure 1), establishing, as it will become clear in the following, a new pseudoaromatic class. These relatively large molecules (molecular mass 736) embody both hydrophilic and hydrophobic groups, which make them easily soluble in solvents like chloroform, acetonitrile and trifluoroethanol, while being sparingly soluble in water. We illustrate here how the coupling process with 1 or 4, by increasing the molecular complexity, gives rise to spectroscopic properties that do not show up with the starting molecules.

Results

Synthesis of the bicolchicides and biisocolchicides

Compounds 2 and 5 could be obtained by homocoupling of chlorococlines by using Semmelhack’s recipe, i.e., by inducing the coupling reaction with stoichiometric amounts of Ni(COD)2 in...
DMF at room temperature [9]. This method is further described in Method S1. As shown in Figure 1A for 10-chlorocolchicide (1) [10] and Figure 1B for 9-chlorocolchicide (4) [10], formation of 2 from the former, and 5 from the latter, was accompanied by hydrogenolysis products, colchicide (3) [11] and isocolchicide (6) [11], respectively. All efforts to carry out these reactions under catalytic conditions with Ni(COD)2 were frustrated. The 1H NMR ddd pattern for H7 and H7' supports pseudoaxial assignment for both protons. Since the synthesis started from (7S)-1, this establishes configuration (R,R',7S,7'S) for compound 2 [12], [13]. For compound 5, 1H NMR spectra at 600 MHz in CDCl3 at r.t. revealed that (R,R',7S,7'S)-biisocolchicide (5a) and (S,S',7'S)-biisocolchicide (5b) equilibrate (83% vs 17%, respectively). These, and all other stereochemical features of these compounds, are dealt with below, step by step, along with spectral evidences.

**Discussion**

**UV and CD spectral behavior of bicolchicides and biisocolchicides**

Figure 2 shows UV and CD spectra in various solvents for the coupling product 2 in comparison with its building block, the hydrogenolysis product 3. It is seen that the UV and CD bands for 2, in the low- and medium-energy spectral range (λ < 300 nm),
undergo a red shift by ca. 50 nm with respect to 3, likely a consequence of conjugation between the two moieties, while gaining in complexity. Extensive conjugation in the dimeric species 2 and 5 is supported by their reduction potential being less cathodic than for the monomeric species 3 and 6, as determined by cyclic voltammetry. Thus, in dried DMF as solvent, vs SCE, cathodic reduction potentials for compounds 2 and 5 turned out to be -1.28 and -1.27 V, against -1.48 and -1.50 V for compounds 3 and 6, respectively. (See Method S2.)

Although the CD bands in the low and medium energy spectral region for both 2 and 3 have the same (negative) sign, they differ vastly in both shape and intensity. Notably, 2 is far more sensitive to the nature of the solvent medium than 3. In the higher energy region (λ<300 nm), the intensity of the CD bands of 2 is even more deeply modulated by the solvent. Thus, with 2,2,2-trifluoroethanol (TFE) and EtOH as solvents, on decreasing the wavelength, the CD of compound 2 attains strongly negative minima, changes its sign at about 240 nm, and acquires positive values at higher energies. In this scenario, the negative and positive CD bands could be considered as branches of an incompletely measured negative exciton couplet. No CD couplet could be observed for 3.

With both the coupling product 5 (which, as demonstrated below, exists as an equilibrium mixture of two atropisomers 5a and 5b, where 5a highly dominates) and the hydrogenolysis product 6, a similar trend is observed in the low- and medium-energy spectral range only (Figure 3). Below 300 nm a positive couplet-like band shows up in alcoholic media like EtOH and TFE. In MeCN and water, the couplet-like nature of the CD band is somewhat blurred, while in CHCl3 and DMSO recording of CD spectra was limited to 240 nm due to strong absorption by the solvents. Remarkably again, the CD with 5 proved far more solvent dependent than with 6.

Such solvent-induced dramatic changes in the CD bands of the coupling products prompted us to investigate in detail the dependence of the spectral changes from the percentage of MeCN in H2O, keeping in mind that the former is a non hydrogen

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**Figure 2. UV and CD spectra for colchicide and bicolchicide.** UV (A) and CD (B) spectra for bicolchicide 2 (solid line) and colchicide 3 (dotted line) in various solvents are reported; ...; H2O; ---: MeCN. doi:10.1371/journal.pone.0010617.g002

**Figure 3. UV and CD spectra for isocolchicide and biisocolchicide.** UV (A) and CD (B) spectra for biisocolchicide 5 (solid line) and isocolchicide 6 (dotted line) in various solvents are reported; ...: EtOH; ---: TFE; ---: H2O; ---: MeCN; ---: CHCl3; ---: DMSO. doi:10.1371/journal.pone.0010617.g003
bonding, polar solvent, while the latter is strongly polar, hydrogen bonding [14]. On mixing these wholly miscible solvents, a contraction of volume occurs, in an endothermic process. Out of the plethora of contrasting interpretations or models introduced to explain particular experimental data [14], this process can be simply interpreted as a collapse of some “icebergs” arising from strong hydrogen bonding between water molecules. CD spectra of 5 at constant concentration in MeCN/H₂O mixtures of nine different compositions - from neat MeCN to neat H₂O - are shown in Figure 4. These spectral modifications cannot be attributed to any change in the [5a]/[5b] atropisomeric equilibrium. Actually, we have proven that the [5a]/[5b] atropisomeric equilibrium is nearly invariant to all compositions of these solvent mixtures, from neat H₂O to neat MeCN: HPLC analysis (see Materials and Methods section) of the above mixtures shows that the percentage of 5a in the atropisomeric mixture remains in the range 83–87% throughout. In the light of these observations, the nine dichroic absorption curves in Figure 4 reveal the presence of two isodichroic points at 320 and 280 nm and seven peaks (“extrema”) that could be localized, at least in some recorded CD spectra, at 377, 342, 305, 270, 252, 235, and 222 nm. The presence of isodichroic points, for solutions at constant molarity of the solute, is taken as an indication that the actual spectra derive from a combination of basic spectra having an identical value at the isodichroic points. Quantitative analysis of the data reported in Figure 4, from neat H₂O to neat MeCN, revealed that the measured CD spectra cannot be expressed as a linear combination of two CD spectra, the one in neat water and the other one in neat MeCN.

The consequences of changing the percentage of H₂O in MeCN can be analyzed by considering the intensity of the CD signal at the wavelengths indicated above. This is shown in Figure 5A and 5B, where the dichroic elongations (mm) are given as a function of the mole fraction of H₂O. The dichroic curves can be grouped into two families. One includes the stronger bands centered at 377, 342 and 235 nm (Figure 5A), where no change in the sign of dichroic bands is observed. In this first family of curves, a very swift change of elongation occurs at vanishingly small concentrations of MeCN, where also thermodynamic ΔH data revealed some peculiarity of the MeCN/H₂O system [15], whilst further additions of MeCN - up to neat MeCN - have relatively scarce effect on the elongations. Such changes in the elongation probably stem from both the well known disruption ability of OH…O hydrogen bonds and the high affinity for the lipophilic parts of the solute by the solvent MeCN. In this scenario, we envisage the presence of two families of conformers, one related to hydrogen bonded structures (Xw > 0.9 in Figure 5A, at Xw = 1) and the other one unrelated to hydrogen bonding (Xw < 0.9). Associating explicit molecular geometries to these frames is a hard task, however, probably beyond the capability of current continuum solvent models. All groups containing oxygen are first candidates for hydrogen bonding. Included are the methoxy groups (in particular the central one in the 1,2,3-trimethoxybenzene moiety, which is significantly more hydrophilic because it is forced out of the plane of the arene ring [15]), the acetylamino groups, and the carbonyl groups.

The negative bands at 377 and 342 nm must arise from partially conjugated electrons of the two cycloheptatrienone moieties, while the 235 nm band can be interpreted as the positive branch of a partially measured CD couplet. All that must stem from identical aromatic transitions, each one localized in the trimethoxybenzene moiety and coupled to each other according to the known exciton
coupling mechanism (see next DeVoe’s model section). In both cases, groups such as methoxy (235 nm band) and carbonyl (377 and 342 nm bands) are present in the moiety which is most involved in the transition, hence most strongly influenced by hydrogen bonding.

The conceivable hypothesis of two structural arrangements, one at \( X_w \approx 1 \) and a second one at \( X_w \approx 0.9 \), from which the stronger CD bands centered at 377, 342 and 235 nm originate, does not find a parallel in the analysis of the second family of curves, the weaker dichroic bands at 305, 270, 252 and 222 nm. These, also in the range \( X_w < 0.9 \), on changing the composition of the solvent undergo marked changes, including the sign of the bands, as it can be appreciated from Figure 5B. More subtly, we are faced by two contrasting observations with these weaker bands. From one side, slight additions of MeCN to aqueous solutions of compound 5 result in steep changes in the elongations, en route to inverting the sign of the bands. From the other side, on adding \( H_2O \) to MeCN solutions of compound 5, a more gradual change in the elongations is observed in comparison with what occurs when \( X_w \) is close to 1. As above, the sign of the bands is reversed.

This is a more complex trend than was observed with the stronger bands in Figure 5A, where at each wavelength-band two limiting bands were observed on changing from neat MeCN to neat \( H_2O \).

Taking into account the low intensity, the close values of frequency with respect to other intense bands, and the complex mechanisms that exist (see infra) for a transfer of rotational strength from one band to another one, no firm conclusion can be reached at this time about the mechanism underlying the spectral changes with these minor CD bands.

DeVoe’s mechanisms for the origin of circular dichroism from asymmetric locations of chromophores

The onset of exciton couplings suggests that the phenomena described in the previous section are amenable to a rationalization on the basis of DeVoe’s classical model [3], [4]. This model is particularly well suited to treat “dimeric” molecules where, like with 2 and 5, chirality is also contributed by deviations from coplanarity of a couple of identical, or nearly identical, quasi-planar chromophores, e.g. the trimethoxy-benzene chromophores. The model can also account for the high sensitivity of CD spectra to relatively minor conformational variations, as it is observed with coupling products 2 and 5, from what is likely a similar effect.

Equation (1), which was derived from a coupled oscillator approach [6], expresses the CD due to a series of transitions 1,2,3,... localized in the chromophores of the molecule and coupled through their dipole-dipole interactions. In this frame, transitions related to strongly conjugated electrons, such as those involved in the \( \lambda > 300 \text{ nm} \) spectral range, cannot be considered.

Equation (1), under simplifying physical conditions, can be used to discuss the higher-energy transitions (\( \lambda < 300 \text{ nm} \)) thanks to an explicit expression of the dependency of \( \Delta \varepsilon \) from both the molecular geometry and the UV spectral features of 1 and 4:

\[
\Delta \varepsilon = 0.0073 \pi^2 N^2 \sum_{i>j} e_i \times e_j R_{ij} \text{Im} A_{ij} \tag{1}
\]

Here, \( \text{Im} A_{ij} \) is the imaginary part of a generic element of matrix \( A \), while \( A \) is the matrix inverse of matrix \( B \), whose generic element \( B_{ij} \) is defined by \( B_{ij} = \delta_{ij} + G_{ij} \).

In this definition, \( G_{ij} \) is the electric polarizability related to the \( i \)-\( j \) electronic transition, while \( G_{ij} \) (Equation [2]) is the interaction energy between two unit point-dipoles \( e_i \) and \( e_j \). These represent the orientation features of dipole transitions i and j, which are localized in different points of the molecule and are interconnected by the distance \( R_{ij} \):

\[
G_{ij} = \frac{e_i \cdot e_j}{R_{ij}^3} - \frac{3 e_i \cdot R_{ij} e_j \cdot R_{ij}}{R_{ij}^5} \tag{2}
\]

Because the polarizability is related to the wavelength, matrix inversion has to be made at each value of \( \lambda \).

A qualitative analysis can be easily carried out under further simplifying conditions, as embodied in Equation (3), which is derived from Equation (1). Equation (3) holds for two identical chromophores with one electrically-allowed transition, in the frame of a treatment first order in the dipole-dipole \( G_{12} \) term [6].

\[
\Delta \varepsilon(v) = C e_1 \times e_2 R_{12} G_{12} v^2 \sigma_1(v) \sigma_2(v) \tag{3}
\]

Here, \( C \) is a constant, \( \sigma_1 \) and \( \sigma_2 \) are the real and the imaginary parts of polarizability \( \sigma_i \) pertaining to the transition “1” and “2”, and \( R_{12} \) is the distance between the two dipoles corresponding to the same electronic transition localized in the two molecular moieties.

Two notable features emerge from Equation (3) for the CD generated at the transition “1” spectral range. The first feature is the typical spectral shape of the couplet, which is due to the product of the imaginary part of the polarizability (the \( \sigma_1(v) \) term) (which acquires maximum value at the wavelength of maximum absorption, \( \lambda_{max} \)) and the real part of the polarizability (the \( \sigma_2(v) \) term).

The latter, for gaussian or lorentzian spectral shape of \( \sigma_1(v) \), undergoes inversion of sign at \( \lambda_{max} \) from positive values at \( \lambda > \lambda_{max} \) to negative values at \( \lambda < \lambda_{max} \), following an asymmetric trend with respect to the \( \lambda_{max} \) position. The second feature that emerges from Equation (3) is that the sign and intensity of \( \Delta \varepsilon \) depend on the molecular geometry, both through the triple mix product of the three vectors that identify the asymmetry of the position/orientation of transition dipoles in the molecule (\( e_1, e_2, R_{12} \)) and the scalar products of vectors present in the energy term \( G_{12} \). Thus, rotation of the two moieties in products 2 and 5 away from a common plane, where \( \Delta \varepsilon = 0 \), gives rise to a positive or a negative couplet; the rate of increase of intensity with the deformation parameter turns out to be proportional to both the square of the molar absorption coefficient of the two interacting identical transitions and the dipolar interaction terms. This means that only strong absorption bands can emerge in the CD spectrum with the typical couplet shape. The \( R_{12} \) distance between the trimethoxy-benzene moieties is also relevant.

It is as high as 14.5 or 13.7 Å for compounds 5a and 5b, respectively, in their most stable conformation, as indicated by DFT minimizations (see the computational section). Thus, because of a \( 1/(R_{12})^3 \) dependence of \( G_{12} \), it is only with particularly favourable orientations of the above three vectors (\( e_1, e_2, R_{12} \)) that the couplet could emerge.

In the presence of a second transition “2”, not overlapping the one in Equation (3), a term expressed by Equation (4) adds to \( \Delta \varepsilon \) from the couplet [7].

\[
\Delta \varepsilon(\lambda) = D_{\Omega 1}(\lambda) e_1 \times e_2 R_{12} G_{12} \tau_2^2(\lambda) \tag{4}
\]

In Equation (4), \( D \) is a constant and “1” represents quantities related to the above described electrically-allowed transition confined to one of the molecular moieties that give rise to the couplet. In turn, “2” represents quantities related to any other transition that does not overlap transition “1” and which is confined to the other molecular moiety. In this case - geometric
factor \( e_1 \times e_2 \ R_{12} \) apart - the contribution of transition "2" to the CD in the spectral zone of transition "1" is proportional to the molar coefficient \( e_1(\lambda) \) and is positive or negative for absorption band "2" lying at shorter or longer wavelength with respect to band "1". Terms of this type may induce a shift of the couplet towards positive or negative values, while also causing a deformation of the couplet itself. Joint action of multiple effects of this type can make very difficult to even recognize the presence of a couplet. Probably multiple effects of this type are at work with compounds 2 and 5, particularly in the case of the bands of Figure 5B, which further complicates the analysis of these bands. When other transitions overlap, even partially, transition "1", difficulties of interpretation reach the apex.

Stereochemical assignments of bicolchicide 2 and bisocolchicide 5, and mutarotation with the minor conformer 5b

Other than in the opposite sign of the couplet in the high-energy CD spectral region - as said above - coupling products 2 and 5 differ dramatically in both HPLC chromatographic and \(^1\)H NMR spectral behavior. While compound 2 showed up, under all circumstances, as a single conformer, the \(^1\)H NMR spectra of compound 5 in CDCl\(_3\) at either 300 or 600 MHz, could only be interpreted by disentangling signals for two conformers, 5a and 5b (Figure 1), in a 4.8:1 peak-area ratio. As HPLC analyses of the mixture gave a similar peak-area ratio for 5a vs 5b (Table 1 and Materials and Methods), the analysis in other solvents was economically carried out from HPLC data alone. The results in Table 1 fail to reveal any trend in the 5a/5b population ratio with the bulk properties of the medium.

While \((R)_{\alpha}7\beta(S)_{\alpha,7'}7\beta\) configurational attribution to 2 and 5a is based on \(^1\)H NMR spectra alone (dddd pattern for H7 and H7' for both 2 and 5a, see Materials and Methods), the stereochemical assignment of the minor conformer 5b was first carried out on the basis of CD spectra. To do so, mixtures containing compound 5 were subjected to HPLC, peak eluates being directly collected into thermostatted CD cuvettes at ca 1°C and rapidly UV and CD analyzed. While the UV spectra proved very similar for all eluates, the dichroism for a minor peak eluted at \( t_R = 8.07 \) min turned out to increase with time, extrapolating at infinite time (ca 24 h) to the dichroism observed for the major peak (\( t_R = 6.62 \) min), as shown in Figure 6. Initially, the dichroism in the area centered at \( \lambda = 350 \) nm was only weakly negative, with elongation much smaller than at infinite time. This is consistent with a partial compensation from the interconnected opposite helices \((R)_{\alpha}7\beta(S)_{\alpha,7'}7\beta\).

Table 1. Experimental distribution of 5a and 5b atropisomers at 300 K.

| Method            | Medium         | \( \Delta^{3 \text{H}} \) | 5b/5a | \( \Delta G_{300} \) (Kcal/mol) |
|-------------------|----------------|---------------------------|-------|-------------------------------|
| \(^1\)H NMR or HPLC | CDCl\(_3\)     | 4.7                        | 17/83 | 0.8                           |
| HPLC              | CH\(_3\)OH      | 24.3                       | 5/95  | 1.7                           |
| HPLC              | C\(_2\)F\(_5\)OH | 26.5                       | 22/78 | 0.8                           |
| HPLC              | CH\(_3\)CN      | 32.6                       | 6/94  | 1.7                           |
| HPLC              | CH\(_3\)SO      | 36.2                       | 13/87 | 1.3                           |
| HPLC              | H\(_2\)O        | 49                         | 3/97  | 2.2                           |
| HPLC              | (CH\(_3\))\(_2\)SO | 78.5                      | 17/83 | 0.8                           |

(a) Dielectric constant.

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Configurational assignment \((R)_{\alpha}7\beta(S)_{\alpha,7'}7\beta\) to 5b was confirmed from \(^1\)H NMR spectra in CDCl\(_3\), which showed two sets of protons for H7 and H7', differing both as to the chemical shift and the coupling pattern. A ddd pattern supports pseudoauxiliary assignment to H7 (i.e. the C7 acetamido group takes a pseudoequatorial position) for the \( R \) part, while a dd pattern supports pseudoequatorial assignment to H7' (i.e. the C7 acetamido group takes a pseudoequatorial position) for the \( S \) part [12], [13].

The time dependency observed for the dichroism of compound 5 must stem from mutarotation, with the minor conformer \((R)_{\alpha}7\beta(S)_{\alpha,7'}7\beta\) undergoing helical inversion to give rise to \((R)_{\alpha}7\beta(S)_{\alpha,7'}7\beta\). The equilibrium constant \( K_{5a,5b} \) between the two atropisomers is sensitive to the solvent nature (Table 1), and, like for the CD spectra of compounds 2 and 5, the changes can hardly be related to the bulk properties of the medium. Moreover, the trend observed for the separate moieties (let them be represented by the known colchicine (7) and isocolchicine (8), Figure 7), is also not followed.
Rapoport and Lavigne first rationalized the mutarotation of 8 in chloroform as due to atropisomeric inversion [16]. They also attributed lack of mutarotation of isocolchicine in ethanol to increased steric bulk of the ethanol-solvated pseudoaxial acetylamo group [16]. Although lack of mutarotation for isocolchicine in ethanol is also consistent with favored solvation of the more exposed pseudoequatorial acetylamo group stabilizing this conformation [17], whichever rationalization applies to this solvent effect, the behavior of the “dimeric” structures, resulting from coupling of either two colchicides, or two isocolchicides, is formally in line with their “monomeric” components. To this concern, it should be noticed that dominance of the (Rα,7S)/(Sa,7'S) atropisomer of isocolchicine in CHCl₃ at r.t. is less than observed (10:1) for the (Sα,7S)/(Sa,7'S) atropisomer in CHCl₃ at r.t. is less than observed (10:1) for the (Sα,7S)/(Sa,7'S) atropisomer of isocolchicine 8 [17]. That is, “dimerization” smoothes out any difference between the equilibrating atropisomers. That said, it should be noticed that the CD spectra of the ensemble (Figure 3B) depend more on solvent effects on conformer 5a than on the position of the equilibrium of this conformer with 5b, in spite of profound differences that exist in the dichroism of the two conformers, 5a and 5b (Figure 6B).

Computational treatment of bicolchicides and biisocolchicides

Structure 5 was minimized in vacuum by both global space search with molecular mechanics [18] and simulated annealing molecular dynamics [19]. Three types of conformers, (Rα,7S)/(Rα,7'S), (Rα,7S)/(Sa,7'S), and (Sa,7S)/(Sa,7'S) emerged. While the latter one is of no interest for its high potential energy, the lowest-energy conformers of the other two types were further minimized by DFT calculations with the M05-2X functional [20] at 6–31G* basis set level (see Calculations S1). The choice of this recent density functional was dictated by its correct treatment - even at this modest basis set level - of the component moieties, colchicine (7) and isocolchicine (8), where the B3LYP density functional resulted in a much too high puckering of the cycloheptatriene ring [17]. Success of M05-2X could be attributed to a better treatment of medium-range correlations than by B3LYP [20]. As shown in Figure 8, with both (Rα,7S)/(Rα,7'S)-5a and (Rα,7S)/(Sa,7'S)-5b the cycloheptatriene ring was simulated correctly by using the M05-2X functional, with only slight puckering in accordance with X-ray diffraction data for colchicinoids [21], [22] or nematic-phase NMR for tropone [23]. According to these DFT calculations, atropisomer 5a is more stable than 5b by 2.5 kcal mol⁻¹. Single point MP2 energy calculations at the same set of basis level, which better account for electron correlations, 5a turned out to be more stable than 5b by a smaller margin, 1.8 kcal mol⁻¹. Still, this is a larger margin than observed experimentally, with the exception of experiments in DMSO as solvent, where calculated values match experimental values (Table 1). However, in view of the lack of any trend of the experimental energy with the solvent properties (Table 1), the agreement observed for DMSO as solvent should be considered as fortuitous. In view of these facts, the coupling product 5 represents a more challenging system than either 8 or 7, where the energy predictions by DFT or MP2 in vacuum are in good agreement with the experimental data in CHCl₃ as solvent [16]. Likely, this reflects the higher complexity of 5.

Quantum mechanical simulation of the UV and CD spectra of atropisomers 5b and 5a was also carried out in the frame of the time-dependent density functional theory (TDDFT), by using BHLYP, a hybrid functional with a large amount of Fock-exchange [24], with TZVP as basis set. Because the RI-DFT mode was used, the auxiliary basis set TZV/J had to be added. It is seen from Figure 9A for atropisomer 5a that the low-energy bands only (λ > 3000) were decently reproduced. It can also be noticed that a plotting common artifice, broadening the CD bands with σ = 0.32, seems to afford a better fitting with respect to narrower bands, e.g. σ = 0.16 [25].
level force field, without any guaranty of success given the tiny spectrum in EtOH,
by three; calculated spectrum in EtOH, conformers by classical MD would require to build an
failed [17]. On the other hand, any treatment of biisocolchicide nucleus, where even the more advanced hybrid functional B3LYP functional [31] is inadequate to treat the cycloheptatrienone molecules of the size of bicolchicides. More fundamentally, the convergence, which would be computationally too costly for
seen in the requirement of trajectories long enough to reach
insertion is carried out on-the-fly. A limitation to this theory can be
[30], or in linear-scaling implementations [31], where parameter-
fundamental theory, such as implemented in the NWChem suite
periodic system, also with pseudopotential plane-wave density
molecules the interplay of various factors in the interaction with circularly polarized light may face such a delicate balance of factors that the presence of the coulplet may even escape attention.

All that draws attention to four key points: (1) the trimethoxy-benzene chromophores are separated by a large distance, ca. 14 Å, (2) they are interconnected by flexible moieties that are exposed to the solvent medium, (3) the CD is highly sensitive both to the relative orientation of these chromophores and (4) the many existing transitions which can modify the coulplet profile. Thus, CD couplets can only emerge clearly when favorable orientations of the transition dipoles are met, which, unsurprisingly, can only happen for certain solvents. All that should be considered in the above illustrated framework, i.e., that for multichromophoric molecules the interplay of various factors in the interaction with our simulations are in accordance for the low-high-energy zone (\(\lambda<300\)), while, like for 5a, the high-energy zone (\(\lambda>300\)) could not be reproduced.

As far as solvent effects are concerned, it must be said that DFT calculations with continuum solvation models, such as devised by Klamt [26], Caricato [27], Cramer and Truhlar [28], and Florián and Waershel [29], do not attain a precision sufficient to deal with the tiny conformational energy differences with atropisomers 5a and 5b and therefore were not even considered for present tasks. MD procedures in explicit solvent could be carried out in a periodic system, also with pseudopotential plane-wave density functional theory, such as implemented in the NWChem suite [30], or in linear-scaling implementations [31], where parameterization is carried out on-the-fly. A limitation to this theory can be seen in the requirement of trajectories long enough to reach convergence, which would be computationally too costly for molecules of the size of bicolchicides. More fundamentally, the currently implemented Becke-Lee-Yang-Parr (B3LYP) density functional [31] is inadequate to treat the cycloheptatrienone nucleus, where even the more advanced hybrid functional B3LYP failed [17]. On the other hand, any treatment of bisocolchicide conformers by classical MD would require to build an ad hoc high-level force field, without any guaranty of success given the tiny energy differences into play.

What remains open to question, are QM-MM calculations, where the solute is treated quantum mechanically, while the solvent is treated classically [32]. A foreseeable problem here is that MD procedures are characterized by extensive sampling in view of long-enough trajectories, in the order of many ns so that, to alleviate the burden of the big matrices, QM-MM procedures of the type required in this work are currently carried out with a semiempirical level for the QM part [32], thus inheriting all limitations of semiempirical treatments. Nonetheless, we tried this route. Thus, PM3 was attempted in explicit solvents with Amber 10 suite and GAFF force field [33], resulting in an unrealistic strong puckering of the cycloheptatrienone rings and unreliable energy output. On the other hand, the SCC-DFTB level [34], attempted in vacuum, led to similarly disappointing results.

Conclusions

A central observation in this work is that DeVoe’s model for circular dichroism [3], [4] is very important. In fact this model allows us to perform a successful qualitative analysis of the dichroic behaviour of both 2 and 5. For any quantitative application of DeVoe’s model [3], [4] prospects are far less alluring, however. In order that the model retains validity in quantitative applications, the electronic transitions on the component moieties of our coupling products should be independent from one another, while with compounds 2 an 5 it is expected that DeVoe’s model is invalidated by substantial conjugation between the component moieties. For the bands at higher energy, in particular those involved in exciton couples, the number, wavelength features, and polarization direction of the transitions need to be accurately known in order that the CD spectrum can be predicted in the frame of DeVoe’s model. This would require a reliable deconvolution of the absorption spectrum for the moieties that constitute the coupling products 2 and 5 into the component bands, as well as the measurement of polarization directions of related transitions. Moreover, the geometrical relationships between the moieties in the global molecule should be accurately known in view of the sensitivity of the CD to even small changes in the relative orientation of transition dipoles localized at each trimethoxy-benzene moiety. These changes may turn out to be cumulative effects of small distortions along the molecular backbone, of near significance for equations (1–4).
adequate models to treat specific interactions of the solvent with organic molecules is another major obstacle, which makes the reliability of computational treatments more and more questionable as the molecule increases in complexity. In our case, on going from colchicines 3 and 6 to coupling products 2 and 5, the borderline between what can be treated and what cannot was surpassed. Our analysis of the CD spectra of “monomers” and “dimers” in various solvents revealed that specific account is needed for each solvent used, a task that we found hard to accomplish. In practice, this borderline in tractability is set by both the characteristics of the CD spectra in solvents of varying properties and by the computational resources. At any event, our study shows that understanding optically active molecules of the complexity of our coupling products needs, from the experimental side, acquiring CD spectra in different solvents, and, from the computational side, establishing and using methodologies able to account for specifically each solvent used.

Our work shows that obstacles faced by the theoretical interpretation of CD spectra get up dramatically. Besides widely documented general difficulties in predicting CD spectra [35], the main asperities at the structural level are identified here in the molecule, where chirality arises more from the helicity than the asymmetric carbon [12]. This is exacerbated, with respect to the molecule, where chirality arises more from the helicity than the asymmetry of the carbon [12]. This is exacerbated, with respect to the molecule, where chirality arises more from the helicity than the asymmetric carbon [12]. This is exacerbated, with respect to the molecule, where chirality arises more from the helicity than the asymmetric carbon [12]. This is exacerbated, with respect to the molecule, where chirality arises more from the helicity than the asymmetric carbon [12]. This is exacerbated, with respect to the molecule, where chirality arises more from the helicity than the asymmetric carbon [12]. 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restored to 17:83 in 150 min at 22 °C. A similar behavior was observed in other solvents, as shown in Table 1. Preparative HPLC collection of eluates at the 6.82 and 8.07 min peaks into precooled cuvettes allowed us to record the CD spectra at ca 1 °C for atropisomers 5a and 5b in MeCN/H2O 40:60 (Figure 7). The CD spectrum of 5b was observed to change with time into a spectrum for the equilibrium 5a/5b mixture, the equilibrium being reached overnight. The initial rate of 5b → 5a transformation could be estimated as ΔCD elongation at 350 nm/Δt = 0.044 cm/min at 1 °C.

Theoretical methods used
Global conformational space search was carried out with program GMMX, based on Steliou’s BAKMDL algorithm, which involves a systematic variation of bond lengths, angles, and formal breaking-closure of rings. Force field MMX [18] was used. Repetitive global space search, forth and back, and from different intermediate positions, converged to the same minima, which should therefore be close to the global minimum. Simulated annealing was carried out with software AMBER with GAFF force field [33], driven by a Python script [19]. DFT calculations were carried out with the suite NWChem [30]. TDDFT calculations were carried out with software ORCA [36]. Analysis and plotting of the results was carried out with software SpecDis v1.45 [25].

Supporting Information

Method S1 Homocoupling of halocohilidices.
Found at: doi:10.1371/journal.pone.0010617.s001 (0.03 MB DOC)

Method S2 Cathodic reduction potentials of colchicine, colchicides and bicolchicides.

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