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Why colchicine does not show mutarotation. With M05-2X density functional in the realm of tricky natural products

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ABSTRACT: It is shown here that density functional theory (DFT) calculations with the recent M05-2X hybrid functional correctly reproduced the conformation of, and energy difference between, (R_a,7*S*)- and (S_a,7*S*)-type atropisomers of isocolchicine. Interconversion of these ^{1}H NMR-observable atropisomers accounts for mutarotation with this compound. In contrast, the classical B3LYP hybrid functional failed to simulate satisfactorily both the geometries and the energies involved. This extends the use of M05-2X to natural products that embody aromatic and flexible pseudoaromatic and saturated rings, as well as *cis/trans* amide chains, which bring on subtle conformational problems. DFT calculations with M05-2X also shed new light on a long-date conundrum in organic chemistry and pharmacology, i.e., why mutarotation was never observed with colchicine. This is now best attributed to a larger energy gap between the (R_a,7*S*) atropisomer and the elusive (S_a,7*S*) atropisomer with this compound. These results solicit a rethinking of the interactions between colchicinoids and proteins. Copyright © 2007 John Wiley & Sons, Ltd.
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KEYWORDS: colchicine; DFT; M05-2X functional; molecular mechanics

INTRODUCTION

The Meadow saffron alkaloid colchicine ((R_a,7*S*)-**1**, Fig. 1) still constitutes the best remedy against severe gout,[1] cutaneous and oral lesions in Behçet's disease,[2] and familial Mediterranean fever.[3] This explains why the risk of cardiotoxicity and multiorgan failure on this drug overdose is accepted.[1] The colchicine scaffold thus continues to attract the attention of drug hunters, as recently with methoxyamine-tethered derivatives, which astonishingly reverse the properties of colchicine from destabilizing to stabilizing tubulin polymerization,[4] like paclitaxel,[5] epothilones,[6] and a few marine metabolites, among which are sarcodictyins.[7]

So much pharmaceutical interest in colchicinoids notwithstanding, knowledge of their structural aspects is far from being exhaustive, though this is a prerequisite to any docking study. What we know is that the C7 chiral carbon contributes modestly to the circular dichroism (CD) of colchicinoids, which is mainly determined by the helicity along the chirality axis C12a–C12b (Fig. 1).[8] For R_a-type colchicine ((R_a,7*S*)-**1**) and isocolchicine ((R_a,7*S*)-**2**, Fig. 1), two conformers–differing only in slight puckering of the cycloheptatrienone ring–have been described from X-ray diffraction analysis of crystals.[9,10] Axial chirality had already been proposed to rationalize the mutarotation of isocolchicine in non-polar solvents via equilibration of (R_a,7*S*)-**2** and (S_a,7*S*)-**2**.[11] Thirty years later, this hypothesis proved correct on observing the coexisting species by high-field ^{1}H NMR in $CDCl_3$ solution at either room temperatures or slightly higher temperatures.[12]

In striking contrast, mutarotation was never observed with colchicine (**1**), and the reasons remained obscure. To this regard, it should be noticed that simple functional group transformations like *N*-methylation or deacetylation are known to suppress mutarotation with isocolchicine,[11] while both atropisomers of 11-aminoisocolchicide proved stable enough to be isolated.[8] In addition, the (R_a,7*S*) and (S_a,7*S*) atropisomers of 8-aminocolchicide could be isolated, probably owing to the stabilizing role of intramolecular hydrogen bonds.[8] To the best of my knowledge, the latter is the sole colchicide derivative for which both (R_a)- and (S_a)-type conformers have been

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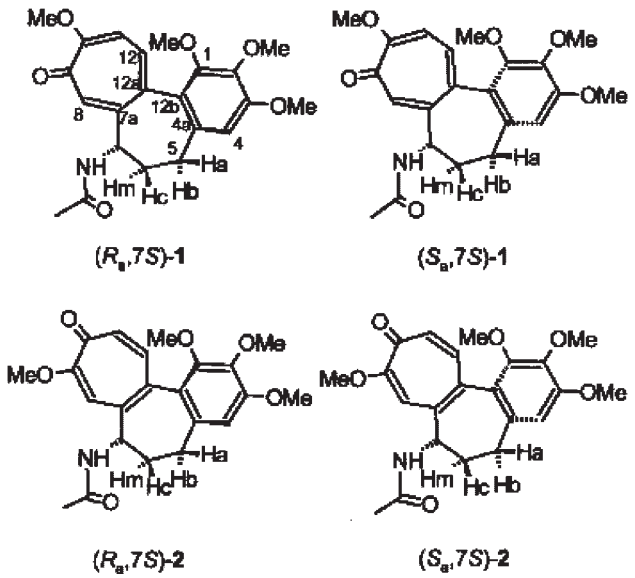

Figure 1. Top: structural representation of (R_a,7*S*)-**1** and hypothetical (S_a,7*S*)-**1** atropisomers of colchicine. Bottom: structural representation of (R_a,7*S*)-**2** and (S_a,7*S*)-**2** atropisomers of isocolchicine

isolated or even detected. All that suggests that a delicate balance of factors must govern equilibria with diastereomeric colchicinoids.

A clarification of the factors that determine conformational equilibria with colchicinoids is thus desirable, and the contrasting behavior of colchicine and isocolchicine illustrated above offers itself as a most appropriate case study. In view of the wealth of chemical and pharmacological studies about colchicine (**1**), incomplete knowledge of its structural features must be imputed to limitations in experimental methodologies. Therefore, to unravel why the (S_a,7*S*) atropisomer of colchicine has remained elusive, recourse to simulation procedures is inescapable. The central question concerns the energies involved in the conformational inversion along the chirality axis 12a–12b, which is required for (R_a,7*S*)-**1** to change into (S_a,7*S*)-**1** (Fig. 1). Modeling these conformers could be approached along various lines. Ideally, the role of the medium should be accounted for. However, current continuum solvation models, such as those devised by Klamt,[13a] Scrocco and Tomasi,[13b] Cramer and Truhlar,[13c] and Florián and Waershel,[13d] fail to attain an accuracy better than 4–5 kcal/mol. Therefore, these methodologies were deemed unsuitable for the present task. On the other hand, free energy evaluation, and exploration of conformational pathways, from classical molecular dynamics is an alluring prospect. a prospect that faces two major obstacles at present, however. One is the nature of the solvent medium, where to model the conformational processes; solvents with such low values of dielectric constant as chloroform–which are of central importance for the colchicinoids (see later)–are difficult to treat, as either implicit or explicit solvent in classical molecular dynamics. Second, extensive high-level re-parameterization of the best-generalized force fields would be required to hopefully account for the tiny energy differences typical of conformational changes with organic molecules. Recourse to *ab initio* molecular dynamics also faces the problem of unbearably high computational cost for molecules as large as the colchicinoids. Finally, it is true that NMR line shape analysis is a powerful approach to the determination of kinetic barriers. It can easily handle simpler systems, for which detailed dynamic ^{1}H NMR experiments have been carried out.[14] This is not the case of colchicine.

METHOD

All QM and GMMX calculations were carried out on a parallel computer based on AMD dual-core Opteron CPUs, with 4 GB RAM per node, driven by Linux Debian amd64 etch as operative system. Program GMMX, version 06, for global conformational space search with MMX, MM3, and MMFF94 force fields,[15] was compiled with gcc 4:4.1.1–15 for serial execution. Conformational search by GMMX is based on Steliou's BAKMDL algorithm, which does a systematic variation of bond lengths, dihedral angles, and formal breaking/reclosure of rings. All GMMX computations were carried out alternatively with the above force fields, and finally with MMX, all pi atoms, energy windows 3.5 and 3.0 kcal/mol for the first and second cycle, respectively. A preliminary VESC calculation to roughly account for pi electrons[16] was carried out.

The MMX force field descends from MM2(77)[17] with the inclusion of VESC pi,[16] and hydrogen bond routines, as well as Still's strategy for generalized parameters.[18] In analogy with MM2, MMX uses bond dipole moments to represent electrostatic contributions, and energies are calculated by taking all dipole–dipole interactions into account. MMX allows to change the value of the dielectric constant used for the calculation of dipolar repulsions. However, this proved of little effect on the calculated structures.

The output structures from GMMX procedures, and collection of Cartesian coordinates for input to the MPQC code,[19] or pdb files for the NWChem code[20] via the ECCE interface,[21] were carried out with the molecular mechanics program PCMODEL, version 9.1,[22] running on Linux Debian i386 etch with OpenGL graphic support. ECCE was also run on Linux Debian i386 etch. The MPQC 2.3.1 program suite,[19] used for DFT/geometry optimization with B3LYP functional, was compiled with gcc 4:4.1.1–15, with libint support, for parallel execution. Geometry optimization was carried out without constraints by a MCSearch OO procedure, which performs extremely efficient line searches with cubic steps, starting from Cartesian coordinates obtained from GMMX

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minimized structures. Default convergence criteria, Max Gradient, Max Displacement, and Gradient Displacement were set at default threshold (0.00001). Convergence for Gradient Displacement was the last to attain, particularly when flat regions were encountered. In such cases the calculation was killed and then restarted from the last good geometry, whereby the guess Hessian was recomputed from this geometry, offering the chance for a better path. All other QM calculations were carried out with the NWChem 5.0 program suite,[20] runtime from Intel® Fortran version 9.1.036, in parallel mode. Using web browsers of the Mozilla family, ECCE allowed control of the QM code via ssh, while permitting remote access to databases via internet. Geometry optimization was carried out without constraints with the NWChem DRIVER module at default convergence criteria, to fit exchange-correlation (XC) potentials. Convergence was always obtained directly, without recourse to any preliminary Hartree–Fock procedure. Møller–Plesset perturbation theory second-order correction to the Hartree-Fock energy (MP2) was carried out single-point on minimized structures with freeze core option. Animation of NWChem vibrational analysis was performed with ECCE. Pdb files, as required by ECCE, were obtained from the GMMX output by PCMODEL.

RESULTS AND DISCUSSION

On the grounds set forth in the Introduction, for an approach to colchicinoids I deemed best to rely on *ab initio* quantum treatments *in vacuum*. Here, the size and complexity of the colchicine molecule leaves room to density functional theory (DFT) only. Although this is considered an *ab initio* procedure, the use of modern, highly parameterized hybrid functionals advised to first validate the methodology with known conformers, using the density functional and basis set of choice. The best possible such test is offered here by the two diastereomers of isocolchicine (**2**, Fig. 1), which differ from colchicine for only having exchanged positions of the carbonyl group and methoxy group at the cycloheptatrienone ring, and for which (R_a, 7*S*)-type conformations are known in the crystalline state.[10] In solution, conformational data for (S_a, 7*S*)-**2** are known only for the C5–C7a region, including the acetylamino side chain, from ^{1}H NMR spectra in $CDCl_3$. Under these conditions, an equilibrium of *ca*. 10:1 ratio in favor of (R_a,7*S*)-**2** was determined at room temperature.[12]

Although DFT procedures are much faster than many-body treatments, a DFT global-space conformational search for the colchicinoids would be prohibitively costly. Perhaps also unwarranted, as classical mechanical theories allow a speedy zero-order approach to the global conformational space problem, while the structures obtained can be later refined by *ab initio* QM procedures in a more restricted conformational space. Under such prospects, the isocolchicine structure was subjected to unbiased search in the global conformational space by the molecular mechanics computer program GMMX,[15] following a preliminary VESC calculation for pi electrons.[16] With MMX force field and a final window of 3 kcal/mol, over 50 conformers were found, lacking any dominant element, i.e., both R_a and S_a helicity could be observed throughout. That least strain-energy structures obtained must be close to the absolute minimum for either helicity type is supported by the consistency of the observations from repetitive global space search, forth and back, and from various intermediate positions. That said, GMMX/MMX attributed (Table 1, last column, second row) a higher relative population to the S_a diastereomer of isocolchicine than observed from ^{1}H NMR spectra in $CDCl_3$[12] (Table 1, last column, first row).[23]

This set the basis for a quantum mechanical approach to geometry optimized isocolchicine. In view of the variety of structural elements involved, the hybrid functional B3LYP, well known for its versatility, was the obvious choice. Calculations were carried out with the MPQC code,[19] input Cartesian geometry provided by the computer program PCMODEL.[22] Poor performance was observed for B3LYP to reproduce both the known geometry of (R_a,7*S*)-**2** in the crystal[9] (Supplementary Material, Table 1S, column 7) and the (S_a,7*S*)-**2**/(R_a,7*S*)-**2** energy ratio in solution,[12] which was computed at an unacceptably larger value than observed experimentally (Table 1, last column, third row).

Disappointed by these results with B3LYP, I set the problem of colchicinoids aside. Reports beginning to appear in the literature on the poor performance of density functionals with respect to both geometry optimization and energy evaluation[24] reinforced my decision. My interest in colchicinoids was only rekindled by the presentation of a new density functional, M05-2X, by Zhao and Truhlar. By better describing medium-range correlations,[25a] this functional was found to account satisfactorily for hydrocarbon geometry and energy

Table 1. Experimental and computed energy difference ΔE (kcal/mol) between (S_a,7*S*)-type and (R_a,7*S*)-type atropisomers of colchicine (**1**) and isocolchicine (**2**)

Method	AO basis	ΔE for **1**	ΔE for **2**
^{1}H NMR			1.0–1.3[a]
GMMX		1.7[b]	1.0[b]
DFT/B3LYP[c]	6-31G*		1.9[d]
DFT/M05-2X[c]	6-31G*	3.5[d], 3.6[e], 3.1[f]	1.2[d]
MP2[g]	6-31G*	3.5[d]	1.3[d]

[a] This represents ΔG, Reference 12.
[b] Strain energy.
[c] Geometry optimization.
[d] 'Equilibrium' energy.
[e] ZPE-corrected, 0 K.
[f] Thermally and ZPE corrected, 298.15 K.
[g] Calculated (single-point) from M05-2X geometry optimized.

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problems,[25b] where all other density functional failed. It performed well also for alkyl bond dissociation energies, transition metal-transition metal, and metal-ligand bond energies, dipole moments in small molecules, as well as donor-acceptor systems, such as HCN-BF_3.[25a] Extension of the use of M05-2X to treat non-covalent complexes such as uracil dimer and pyrazine dimer, as well as other complexes between fragments of biological relevance, has also been extensively documented.[26]

DFT geometry optimization of isocolchicine with the M05-2X functional, starting from the minimum strain-energy structures described above from GMMX, was carried out with the NWChem code.[20] Input was provided by PCMODEL[22] as pdb files given to the ECCE interface.[21] The optimized geometries are shown in Fig. 2 , top for (R_a,7*S*)-**2** and Fig. 2, bottom, for (S_a,7*S*)-**2**.

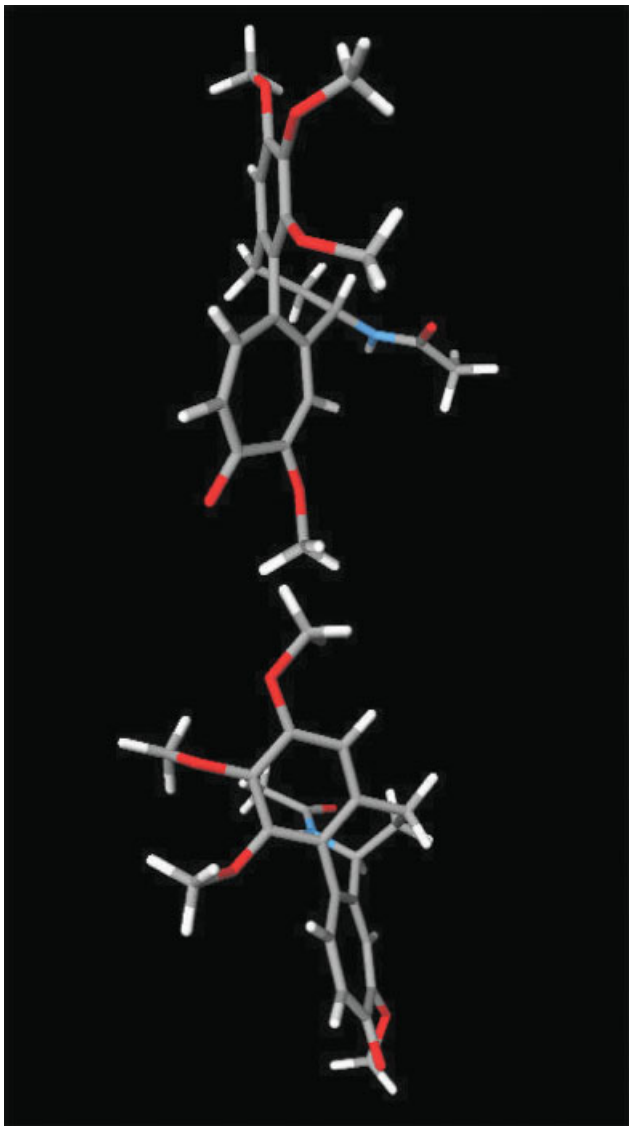

Figure 2. Geometry optimized structure (level DFT/M05-2X/6-31G*) of isocolchicine atropisomers. Top: (R_a,7*S*)-**2** atropisomer. Bottom (S_a,7*S*)-**2** atropisomer.

Key comparative geometric data are summarized in Table 1S of Supplementary Material. From these data, nice agreement can be appreciated with both X-ray diffraction data for (R_a,7*S*)-**2**[10] and ^{1}H NMR data for both (R_a,7*S*)-**2** and (S_a,7*S*)-**2**.[12] The spatial arrangement of the methoxy groups at C1 and C2 (atom numbering in Fig. 1) in the aromatic ring warrants notice. Their *syn* disposition toward the acetylamino group, in minimized (R_a,7*S*)-**2** (top structure), corresponds to 'molecule b' in the crystal.[10] In 'molecule a' in the crystal[10] the methoxy group at C2 points away from the acetylamino group. In minimized (S_a,7*S*)-**2** (bottom structure), the methoxy groups at C2 and C1 are *anti* to one another, with C1 pointing away from the acetylamino group, in agreement with dihedral angles evaluated from ^{1}H NMR in $CDCl_3$ solution.[12]

A good agreement of energies from DFT calculations with single-point Møller–Plesset perturbation theory (MP2) calculations at the same basis set level can be appreciated from Table 1.

These results extend the validity of the M05-2X functional to structurally and conformationally tricky natural products. Excellent performance with colchicinoids is remarkable for a highly parametrized functional like M05-2X, and without recourse to 4n-f orbitals of previous studies,[25,26] which would be computationally too costly for compounds of the size and complexity of the colchicinoids. Thus, the new M05-2X density functional defeated molecules that embody a variety of functional groups with subtle conformational problems, like invertible helicity along a chirality axis, *cis/trans* arrangement of amides, as well as aromatic and pseudoaromatic rings. As to the latter, the agreement of slight ring puckering of the cycloheptatrienone ring for minimized isocolchicine with X-ray diffraction data[9–10] (Table 1S in Supplementary Material) is noticeable. In view of the high degree of planarity of tropone,[27] it seems that puckering of the cycloheptatrienone ring with isocolchicine stems from alleviating repulsions between the carbonyl carbon and the methoxy group as well as tensions in the doubly fused seven-membered central ring.

Agreement between modeling and experiments for isocolchicine justified attacking the problem of the elusive diastereomer (S_a,7*S*)-**1** of colchicine along similar lines. DFT/M05-2X/6-31G* geometry optimized (R_a,7*S*)-**1** turned out to be in agreement with X-ray diffraction data for the crystal (Supplementary Material, Table 2S). The only noticeable difference concerns the methoxy groups at C1 and C2 (atom numbering in Fig. 1), which are *syn* in the crystal for both 'molecule a' and 'molecule b', pointing toward the acetylamino group.[9] In minimized (R_a,7*S*)-**1** (Figure 3, top), these two methoxy groups are *anti* to one another, the one at C2 pointing away from the acetylamino group. In the elusive (S_a,7*S*)-**1** atropisomer, the relative disposition of the C2 and C1 methoxy groups is also *anti*, with the latter pointing away

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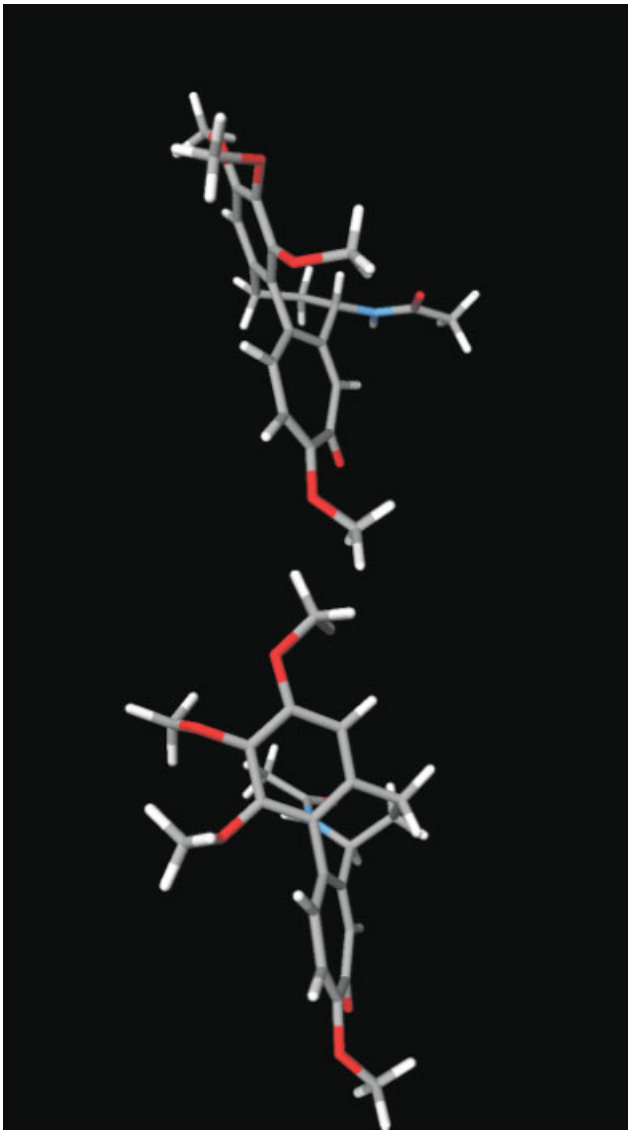

Figure 3. Geometry optimized structure (level DFT/M05-2X/6-31G*) of colchicine. Top (R_a,7*S*)-**1** atropisomer. Bottom: hypothetical (S_a,7*S*)-**1** atropisomer.

from the acetylamino group (Figure 3, bothom), arguably to release strain, as in the isocolchicine analogue above (S_a,7*S*)-**2**.

According to these calculations the (S_a,7*S*)-**1** atropisomer is higher enough in energy with respect to the (R_a,7*S*)-**1** atropisomer to be scarcely populated. However, these are 'equilibrium' energies for situations that cannot be attained in practice. Therefore, a frequency calculation to correct for both zero-point energy (ZPE) and thermal contributions was in order. The results are shown in Table 1 (and, in detail, in the Supplementary Material, Table 3S). By applying thermal correction (which includes ZPE correction), it is calculated that (R_a,7*S*)-**2** is favored over (S_a,7*S*)-**2** by 3.1 kcal/mol. This rationalizes why neither mutarotation for colchicine, nor direct observation of (S_a,7*S*)-**1**, have ever been recorded in any kind of medium. Under normal laboratory conditions, diastereomer (S_a,7*S*)-**2** cannot weight enough. It should be noticed that ZPE and thermal correction did not change sizably the values of 'equilibrium' energies (A reviewer has suggested an alternative explanation for the S_a stereoisomer of colchicine not being observable. The reviewer suggested that with colchicine there is a higher kinetic barrier to atropisomer interconversion than with isocolchicine. Actually, this is the preferred rationalization in the original paper by Rapoport and Lavigne, who wrote 'The absence of mutarotation with colchicine might be due to greater rigidity in this molecule, which seems to be supported by a study of models, and hence a significantly higher activation energy'.[11] I disfavor this view. In the years elapsed since Rapoport and Lavigne work,[11] colchicine was subjected to so many thermal treatments, during synthetic work, with recrystallization from hot solvents. This offered any chance for the S_a atropisomer to become sizably populated. On cooling, a high kinetic barrier would have prevented the S_a atropisomer to revert quickly to the R_a atropisomer. Even if the energy difference between the two was in the same range as for isocolchicine, seemingly similar samples of colchicine would have given different specific optical rotation. This was never reported.

Rapoport and Lavigne further argued in support of their kinetic rationalization that 'The lack of mutarotation [of isocolchicine] in ethanol might be explicable on the basis of solvation of the acetamido group. This would increase its effective size enough to increase the activation energy and lead to only one diastereomer in solution. Deacetylation might decrease the size of this group sufficiently to lower the activation energy and make mutarotation unobservable at room temperature'.[11] In my alternative, thermodynamic rationalization, the acetamido chain may be seen to be more exposed to solvation in the pseudoequatorial than the pseudoaxial diastereomer. This would determine preferential solvation by ethanol of the pseudoequatorial acetamido group, thus favoring this conformer. On the other hand, deacetylation leaves a strongly basic amino group, where the argument of stabilization by solvation of the pseudoequatorial conformer applies forcefully. Nonetheless, I agree that a high-level, computationally very demanding QM or MD study of the conformational pathway for colchicine would shed further light on the behavior of this important class of compounds, and would remove any remaining reasonable doubt.). This justifies my neglecting of such lengthy corrections for isocolchicine, where the same trend is expected. Extremes in the normal modes of vibration (as illustrated for isocolchicine in Supplementary Material) range from the aromatic moiety being closest to the acetylamino chain, to being far apart from it. Actually, for atropisomer (R_a,7*S*)-1 the C1-OMe methyl group moves toward, an back away from, the acetylamino oxygen (Supplementary Material, Figure 1aS and 1bS, respectively), while with atropisomer (S_a,7*S*)-1 the C2-OMe methyl group moves toward, and back away

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from, the acetylamino methyl group (Supplementary Material, Figure 2aS and 2bS, respectively). These forth and back movements are accompanied by smaller amplitude movements of the whole skeletal framework, in a sort of 'breathing' of the molecule. This should be taken into account when examining models for the interaction of colchicine with tubulin.

SUPPORTING INFORMATION

Supporting information: (i) Cartesian coordinates for all molecules involved in this paper, (ii) geometrical data for DFT geometry optimized conformers of both isocolchicine (Table 1S) and colchicine (Table 2S), with added, when available from literature, comparative geometrical data from X-ray diffraction or ^{1}H NMR spectra, (iii) structural representation of the normal vibration modes for both R_a,7*S* colchicine (Figs 1aS/1bS) and S_a,7*S* colchicine (Figs 2aS/2bS).

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