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Vibrational Circular Dichroism Analysis Reveals a Conformational Change of the Baccatin III Ring of Paclitaxel: Visualization of Conformations Using a New Code for Structure−Activity Relationships

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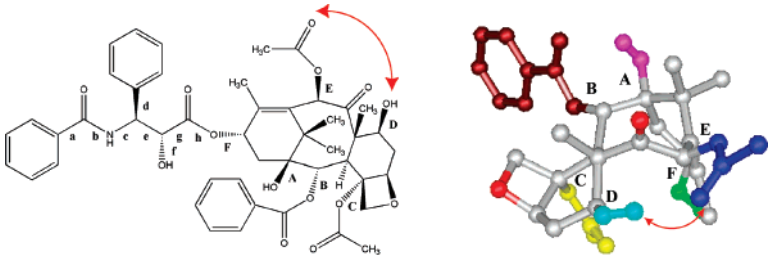

The comparison between measured and conformer-weighted calculated VCD spectra of the baccatin III ring of paclitaxel and visualization of the conformations using the new code for structure−activity relationships are reported for the first time. The VCD spectrum of paclitaxel closely resembles that of the baccatin III ring. The large characteristic ν_{CO} VCD bands with bisignate signs (1732 cm^{-1}, $\Delta\epsilon = -1.6 \times 10^{-1}$; 1715 cm^{-1}, $\Delta\epsilon = 2.4 \times 10^{-1}$) strongly reflect the structural property of the family of conformations ***bacc*-ABC32F** defined using the new code. The comparison with the conformation of the baccatin III core in the electron micrograph of the crystal structure of tubulin−paclitaxel (1JFF) suggests a conformational change of paclitaxel corresponding to a switch through the binding with β-tublin and the intermolecular interactions involving the hydroxyl group (**D**) and carbonyl of acetoxy group (**E**). The representation of conformational codes allows complicated conformations to be very easily compared and facilitates future computational analyses such as those for the large-molecule calculations as well as genome analysis.

Introduction

The determination of conformations of a ligand at its binding site within a protein is important in view of the design and modeling of action of pharmaceutical compounds.[1] However, at their binding sites, the flexibility of active ligands allows them to deform easily. The deformation seems to be especially significant for the metabolism of pharmaceutical compounds because a tightly bound protein−ligand complex works toxically. In this context, it is also important to determine the relationship between the conformations of the ligand bound in the protein complex and free in solution.

We have used vibrational circular dichroism (VCD) spectroscopy[2] to determine the absolute configuration and conformation of free ligands in the solution state. Recently, we have shown that VCD spectroscopy is highly sensitive to the conformations of chiral molecules and that the well-known odd−even effect of chiral alcohols in the solution state can be

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(1) (a) Alcaraz, A. A.; Mehta, A. K.; Johnson, S. A.; Snyder, J. P. *J. Med. Chem.* **2006**, *49*, 2478−2488. (b) Tang, S.; Yang, C.; Brodie, P.; Bane, S.; Ravindra, R.; Sharma, S.; Jiang, Y.; Snyder, J. P.; Kingston, D. G. I. *Org. Lett.* **2006**, *8*, 3983−3986. (c) Sánchez-Pedregal, V. M.; Kubicek, K.; Meiler, J.; Lyothier, I.; Paterson, I.; Carlomagno, T. *Angew. Chem., Int. Ed.* **2006**, *45*, 7388−7394. (d) Johnson, S. A.; Alcaraz, A. A.; Snyder, J. P. *Org. Lett.* **2005**, *7*, 5549−5552.

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theoretically explained using VCD spectra, deduced conformations, and associated vibrational mode patterns.[3] We have also indicated that a fliplike motion of thalidomide dimers can be deduced from their VCD spectra in $CDCl_3$ and DMSO-d_6.[4] From these studies, we have determined the importance of identifying the major solution-state conformations of flexible chiral molecules in order to assign their absolute configuration. Following this work, we have used VCD spectroscopy to undertake the conformational analysis of the baccatin III ring, which forms the chiral core of the anticancer drug paclitaxel (Taxol). These studies have yielded new information about the relationship between the predominant conformations of free paclitaxel in the solution state and the bioactive conformation of this important drug molecule at its active site.

In the case of paclitaxel in complex with β-tublin, several bioactive conformations (T-Taxol, PTX-NY, REDOR, Polar, and 1JFF) have been proposed.[1a] Unfortunately it is not possible to visualize these structures from their names alone, nor is it easy to compare conformations using only Cartesian coordinates and the Z-matrix based on their names alone. In carrying out the present study, we have introduced an expansion of IUPAC nomenclature[5] to describe the conformations of large molecules using a new conformational code. In this paper, we describe the conformational change of the baccatin III ring containing a key hydrogen bond that undergoes a conformational switch upon binding with β-tublin as deduced from the comparison with the previously proposed 1JFF conformation of the same ring,[6] the only other readily accessible detailed conformation of this important ring structure. We also present the details of this conformational code that enables the conformations of complex molecules to be visualized easily from their names.

Results and Discussion

Conformational Code. According to IUPAC nomenclature, a conformational element can be defined with respect to a dihedral angle such as shown in Figure 1a.[5] The terminal atom of the corresponding center bond for the determination of dihedral angle is connected to several groups or atoms. The priority rule of the selected group or atom for the determination of dihedral angle applies the IUPAC Rules for Nomenclature of Organic Chemistry, Rule E-5.6.[5] That is to say, if all the groups or atoms are different, Rule E 4.9 for the definition of chirality *R* or *S* is applied correspondingly, and the group or atom with the most prior order is selected for the determination of dihedral angle.[5] For example, in the case of EtMe(HO)C−, the hydroxyl group is selected. If only one group or atom is different and all the other groups or atoms are the same, the unique one is selected. For example, in the case of Et_2MeC-, the methyl group is selected. If all the groups or atoms are the same (for example, in the case of Et_3C-), these (Et) cannot be distinguished. In that case, the group or atom with the smallest torsion angle is selected.

These conformational elements can be digitized as follows: *ap* (+*ap* and −*ap*) = 1, +*sc* = 2, −*sc* = 3, *sp* (+*sp* and −*sp*) = 4, +*ac* = 5, −*ac* = 6, +*ap* = 1β, −*ap* = 1α, +*sp* = 4α, and −*sp* = 4β (α = clockwise and β = counterclockwise). The usual conformational terms, *trans*, +*gauche*, and −*gauche*, correspond to *ap* (1), +*sc* (2), and −*sc* (3), respectively. Usually, most of the conformations are defined by using the conformational elements 1−6. For example, at the position of the hydroxyl group (**D**) in Figure 1b, the O−H and CH−CCO bonds are selected from the above priority rule of the selected group or atom for the determination of dihedral angle. In the case of Figure 2a, the dihedral angle between these groups belongs to the −*sc* region, and then the conformational element becomes 3. On the other hand, at the position of the acetoxy group (**E**) in Figure 1b, the O−CO and CH−CO bonds are selected. In the case of Figure 2b, the dihedral angle between these groups belongs to the +*sc* region, and then the conformational element becomes 2. Occasionally, as for *ap* (1) and *sp* (4), 2 conformations are optimized in the same region by theoretical calculations. In such cases, only the conformational elements 1β (+*ap*), 1α (−*ap*), 4α (+*sp*), and 4β (−*sp*) are used. In this manner, the recognition of different conformational elements having a range of slight diversity, which should be essentially categorized as the same region such as *trans*, can be avoided. For example, at the position of the benzoylamino group (**a**) in Figure 1c, 2 conformations are optimized, and the conformational elements 4α and 4β exist (Figure 2c).

Second, angle locations are represented according to the following description: [***prefix of ring***]-[**angle locations of ring**]-[**the first angle location of connection between ring and side chain**]([***prefix of side chain***]-[**angle locations of side chain**])[**second angle location of connection between ring and side chain**]([***prefix of side chain***]-[**angle locations of side chain**])[**third angle location of connection between ring and side chain**]([***prefix of side chain***]-[**angle locations of side chain**])].... The order of the angle locations corresponds to the numbering of the compounds. For example, in Figure 1b, the C1 and C13 carbons correspond to the angle locations **A** and **F**, respectively. At the connection part between ring and side chain, two side chains can be bound, and the priority rule of the side chain also correspondingly applies by the above Rule E-5.6.[5] The angle locations of the side chain are defined from the terminal building block in order, and prefixes of building blocks are also used, if necessary. At the angle locations of the side chain, the above priority rule (Rule E-5.6) is also applied correspondingly.

Finally, after the omission of useless prefixes and angle locations, the conformational representation of the entire molecule is specified by the final code. In the case of baccatin III (prefix ***bacc***), the angle locations of the ring are omitted

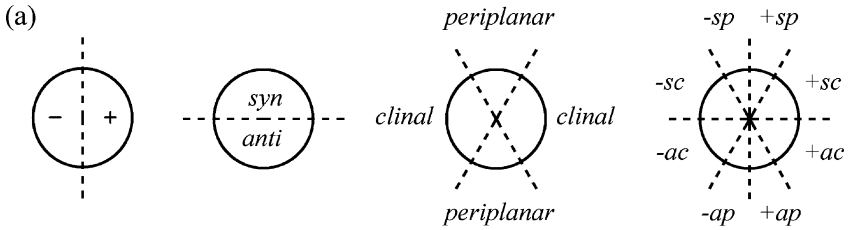
(2) (a) Gautier, C.; B Bürgi, T. *J. Am. Chem. Soc.* **2006**, *128*, 11079−11087. (b) Kuppens, T.; Herrebout, W.; van der Veken, B.; Bultinck, P. *J. Phys. Chem. A* **2006**, *110*, 10191−10200. (c) Naubron, J.-V.; Giordano, L.; Fotiadu, F.; Bürgi, T.; Vanthuyne, N.; Roussel, C.; Buono, G. *J. Org. Chem.* **2006**, *71*, 5586−5593. (d) Monde, K.; Miura, N.; Hashimoto, M.; Taniguchi, T.; Inabe, T. *J. Am. Chem. Soc.* **2006**, *128*, 6000−6001. (e) Brotin, T.; Cavagnat, D.; Dutasta, J.-P.; Buffeteau, T. *J. Am. Chem. Soc.* **2006**, *128*, 5533−5540. (f) Vargas, A.; Bonalumi, N.; Ferri, D.; Baiker, A. *J. Phys. Chem. A* **2006**, *110*, 1118−1127. (g) Furo, T.; Mori, T.; Wada, T.; Inoue, Y. *J. Am. Chem. Soc.* **2005**, *127*, 8242−8243. (h) Izumi, H.; Futamura, S.; Nafie, L. A.; Dukor, R. K. *Chem. Rec.* **2003**, *3*, 112−119. (i) Freedman, T. B.; Cao, X. L.; Dukor, R. K.; Nafie, L. A. *Chirality* **2003**, *15*, 743−758. (j) Wang, F.; Wang, Y.; Polavarapu, P. L.; Li, T.; Drabowicz, J.; Pietrusiewicz, K. M.; Zygo, K. *J. Org. Chem.* **2002**, *67*, 6539−6541. (k) Cheeseman, J. R.; Frisch, M. J.; Devlin, F. J.; Stephens, P. J. *Chem. Phys. Lett.* **1996**, *252*, 211−220.

(3) Izumi, H.; Yamagami, S.; Futamura, S.; Nafie, L. A.; Dukor, R. K. *J. Am. Chem. Soc.* **2004**, *126*, 194−198.

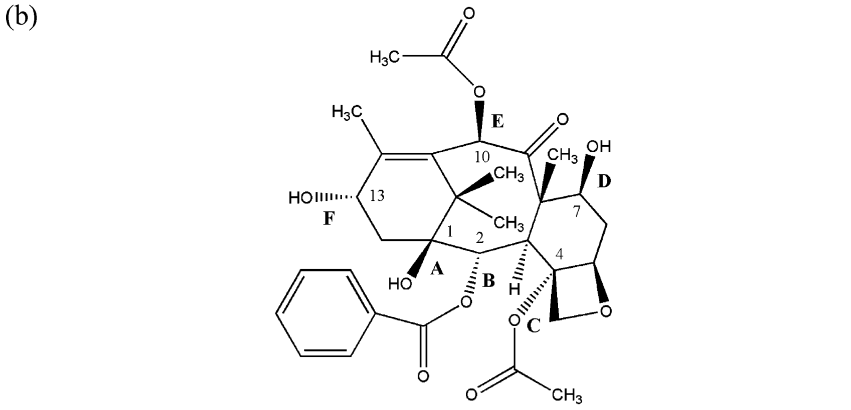
(4) Izumi, H.; Futamura, S.; Tokita, N.; Hamada, Y. *J. Org. Chem.* **2007**, *72*, 277−279.

(5) (a) Cross, L. C.; Klyne, W. *Pure Appl. Chem.* **1976**, *45*, 11−30. (b) International Union of Pure and Applied Chemistry, Organic Chemistry Division, Commission on Nomenclature of Organic Chemistry. In *Nomenclature of Organic Chemistry, Sections A, B, C, D, E, F,* and *H*; Rigaudy, J., Klesney, S., Eds.; Pergamon Press: Oxford, UK, 1979.

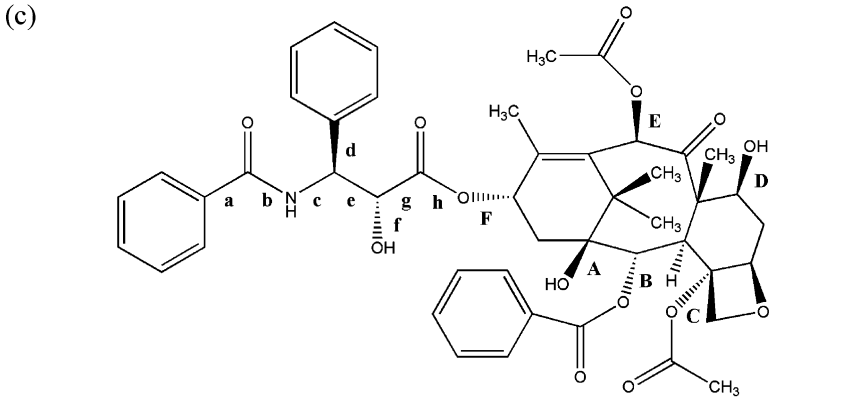
(6) Löwe, J.; Li, H.; Downing, K. H.; Nogales, E. *J. Mol. Biol.* **2001**, *313*, 1045−1057.



ap = 1, +*sc* = 2, -*sc* = 3, *sp* = 4, +*ac* = 5, -*ac* = 6, +*ap* = 1 β, -*ap* = 1 α, +*sp* = 4 α, -*sp* = 4 β



***bacc*-ABCDEF**



***bacc*-ABCDEF(*tail*-abcdefgh)**

FIGURE 1. (a) Classification of dihedral angles for conformational representation according to IUPAC nomenclature. (b) Chemical structure and representation of conformational codes for baccatin III. (c) Chemical structure and representation of conformational codes for paclitaxel.

because only one conformation is optimized for the ring structure. At the position of the acetoxy group (**E**) in Figure 1b, the prefix and angle locations of the side chain are omitted because the population of one conformation is extremely large for the acetoxy group. The conformations are eventually represented as ***bacc*-ABCDEF** where **A**, **B**, **C**, **D**, **E**, and **F** are angle locations for conformational codes defined above (Figure 1b). Further, the conformations of paclitaxel are represented as ***bacc*-ABCDEF(*tail*-abcdefgh)** similarly (Figure 1c). For example, the conformation of paclitaxel for 1JFF[6] is represented as ***bacc*-A33D53(*tail*-24623f14)** because of the omission of hydrogen atoms.

VCD Analysis of Paclitaxel and Baccatin III Using Conformational Code. Figure 3a shows the VCD and IR spectra of paclitaxel and baccatin III ($CDCl_3$, 0.029 M, BaF_2, 72 μm path length). Both VCD spectra closely resemble each other and reflect the rigid structure of the baccatin III skeleton. Large characteristic ν_{CO} VCD bands with bisignate signs (1732 cm^{-1}, $\Delta\epsilon = -1.6 \times 10^{-1}$; 1715 cm^{-1}, $\Delta\epsilon = 2.4 \times 10^{-1}$) were observed for baccatin III. To assign these bands, conformational and vibrational analyses of baccatin III using conformational codes were carried out using density functional theory calculations.[7] Following a random conformational search, 23 lowest energy conformations were selected.[8] Finally, these were reduced to 13 conformations, and the 3 conformations ***bacc*-233323**, ***bacc*-233322**, and ***bacc*-233321** accounted for greater than 97% of the calculated population distribution.[8] The difference between the 3 conformations was the direction of the hydroxyl group at the angle location **F**, at which the tail part of paclitaxel is connected. The measured VCD spectrum of baccatin III was found to be in good agreement with the population weighted VCD spectrum of the 3 energetically

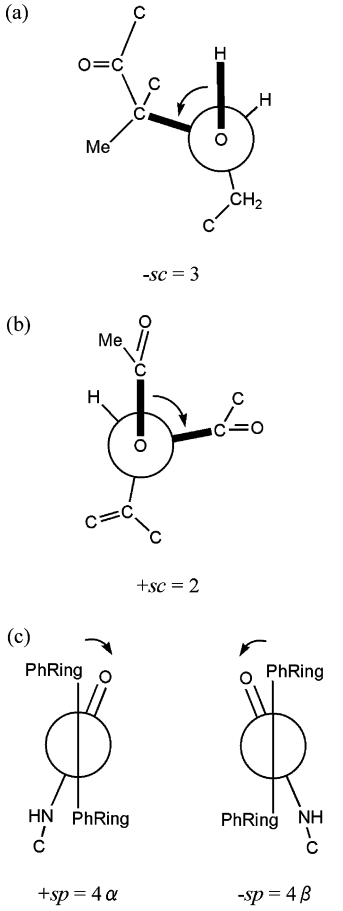


FIGURE 2. (a) Dihedral angle at angle location of the hydroxyl group (**D**). (b) Dihedral angle at angle location of the acetoxy group (**E**). (c) Dihedral angles at angle location of the benzoylamino group (**a**).

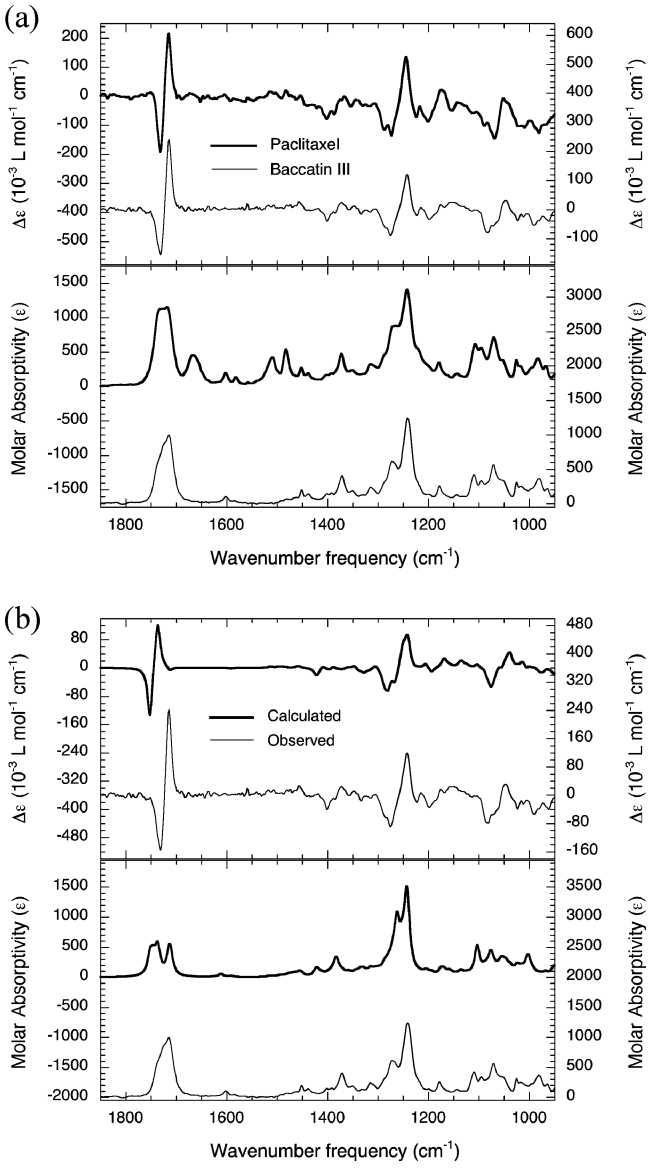


FIGURE 3. (a) Comparison of the measured VCD ($\Delta\epsilon$) and IR (ϵ) spectra of paclitaxel ($CDCl_3$, 0.029 M, BaF_2, 72 μm path length) with the measured VCD ($\Delta\epsilon$) and IR (ϵ) spectra of baccatin III ($CDCl_3$, 0.029 M, BaF_2, 72 μm path length). (b) Comparison of the measured VCD ($\Delta\epsilon$) and IR (ϵ) spectra of baccatin III ($CDCl_3$, 0.029 M, BaF_2, 72 μm path length) with the predicted (population weighted) spectra of three energetically preferred conformations for baccatin III (B3LYP/6-31G*).

preferred conformations for baccatin III (Figure 3b). On the other hand, the measured ν_{CO} IR bands of baccatin III did not correspond as closely to the population weighted IR bands of the 3 energetically preferred conformations. Careful consideration to contributions of the other many conformations including the solvent effect may be necessary, or the lack of agreement may be simply due to the inadequacy of the choice of DFT functional and basis set to predict accurately the frequency spacings and intensities of the vibrational modes in the ν_{CO} IR bands. It is well-known that the B3LYP functional may not represent frequencies well that involve hydrogen bonding (see below for discussion on hydrogen bonding in the calculated conformer structures). In any case, these findings suggest that the calculation of the minor conformers representing less than 3% of the calculated population is not needed for the reproducibility of theoretical VCD spectra.

Further, correlations between each conformation and its VCD spectrum were investigated. Predicted ν_{CO} VCD bands of conformation ***bacc*-233323** (Figure 4a) also corresponded well to those observed for baccatin III, but the unstable conformations ***bacc*-233253** (Figure 4b) and ***bacc*-233133** (Figure 4c) did not at all. The ν_{CO} VCD bands of baccatin III strongly reflected the structural property of conformations ***bacc*-ABC32F** and were assigned as the vibrational modes of carbonyl in the acetoxy group (**E**) and the proximate ring carbonyl group.[8] From these results, it is suggested that the hydroxyl group (**D**) and the

(7) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, Revision B.04; Gaussian, Inc.: Pittsburgh, PA, 2003.

(8) See the Supporting Information.

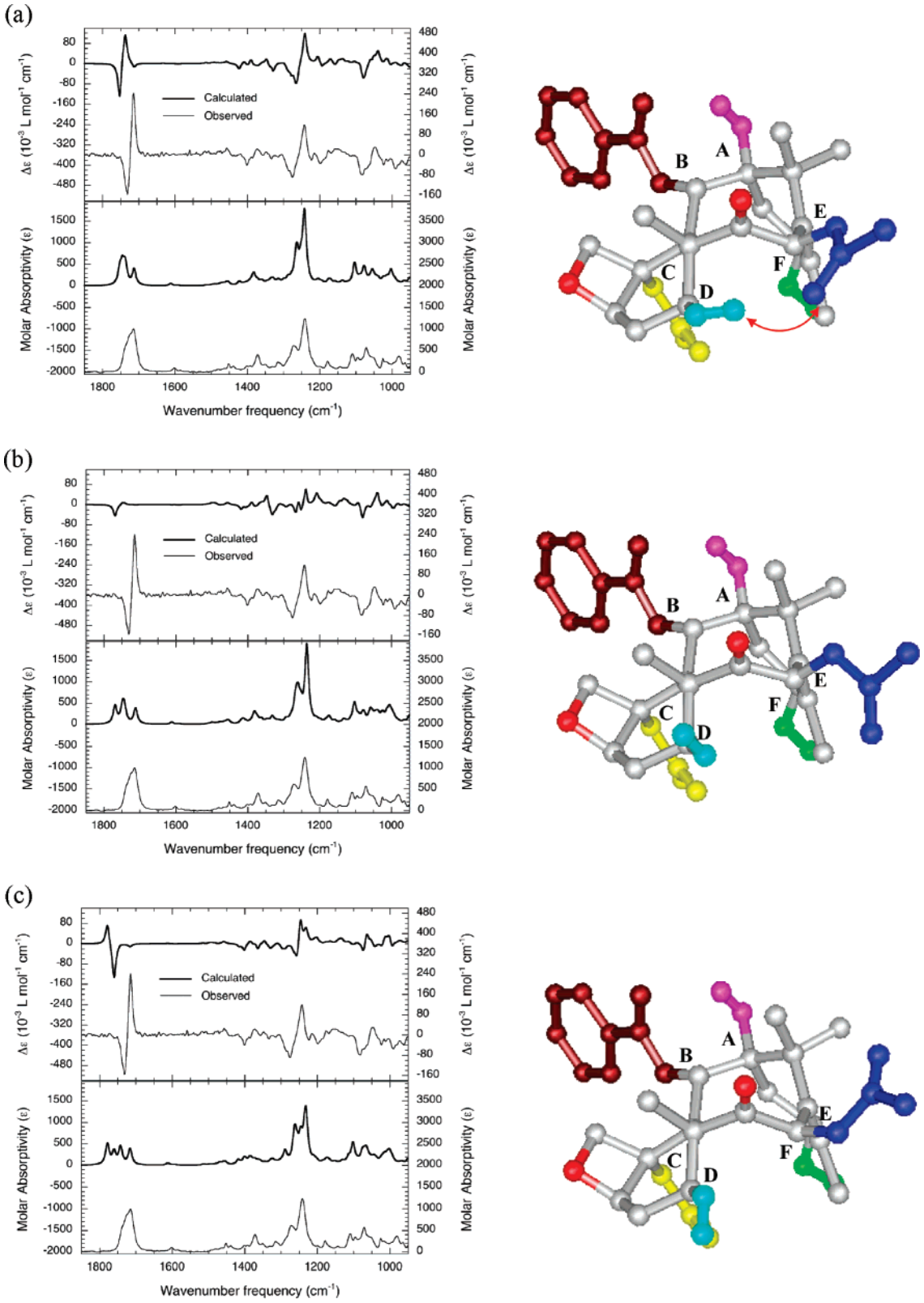


FIGURE 4. Comparisons of the measured VCD ($\Delta\epsilon$) and IR (ϵ) spectra of baccatin III ($CDCl_3$, 0.029 M, BaF_2, 72 μm path length) with the predicted spectra (left) and the optimized geometries (right) of conformations (a) ***bacc*-233323**, (b) ***bacc*-233253**, and (c) ***bacc*-233133** (B3LYP/6-31G*). Hydrogens (except for hydroxyl groups) are omitted.

carbonyl of the acetoxy group (**E**) in ***bacc*-ABC32F** are strongly hydrogen bonded (Figure 4a). For the 1JFF conformation in the electron crystallographic tubulin−paclitaxel structure,[6] the conformation of the baccatin III core is represented as ***bacc*-A33D53** and resembles the unstable conformation ***bacc*-233253**.[8] This finding suggests a conformational change of paclitaxel upon

binding with β-tublin involving the intermolecular interaction of the hydroxyl group (**D**) and the carbonyl of the acetoxy group (**E**). For NMR and crystallographic studies, a translation of spectral data into distance functions is necessary to compare with the theoretical predictions. VCD spectroscopy, on the other hand, can directly connect with the theoretical predictions via the comparison of measured and simulated spectra.

Conclusions

In the present study, we investigate the comparison between conformer population-weighted predicted VCD spectra and the experimental spectra of the baccatin III ring of paclitaxel using a new conformational code for structure−activity relationships. The measured VCD spectrum of baccatin III corresponds to the population-weighted VCD spectrum of the 3 energetically preferred conformations ***bacc*-233323**, ***bacc*-233322**, and ***bacc*-233321**. The important point is that the contributions of minor conformers representing less than 3% of the calculated population are not needed for the reproducibility of theoretical VCD spectra. The comparison with the conformation of the baccatin III ring core in the crystallographic tubulin−paclitaxel structure (1JFF)[6] suggests a conformational change in paclitaxel upon binding with β-tublin involving the intermolecular interaction of the hydroxyl group (**D**) and carbonyl of acetoxy group (**E**). The new method of conformational codes allows complicated conformations to be very easily compared, and the method also can be applied to the large-scale computation as well as genome analysis. These codes are also useful in the visualization of solution conformations for the determination of absolute configuration.

Experimental Section

Measurements. All reagents were of commercial grade. The infrared and VCD spectra were recorded on a commercial Fourier transform VCD spectrometer. The VCD spectra of the solution state were recorded with 4−5 h data collection time at 4 cm^{-1} resolution. The $CDCl_3$ solutions were placed in a 72 μm path length cell with BaF_2 windows. In the VCD spectra of the solution state, the raw VCD spectra of the solvents were subtracted.

Calculations. All geometry optimizations, conformer searches, vibrational frequencies, and absorption and VCD intensities for baccatin III were calculated with use of the Gaussian 03 program[7] on a Pentium 4 (3.2 GHz) PC. Density functional theory with B3LYP functional and 6-31G(d) basis set was used for the calculations. The theoretical absorption and VCD spectra were simulated with Lorentzian band shapes and 6 cm^{-1} full width at half-height. The ab initio frequencies were scaled by 0.97, and the thermal corrections to Gibbs free energies were scaled by 0.9989.

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Supporting Information Available: Relative Gibbs free energies and populations of conformations for baccatin III; Cartesian coordinates of ***bacc*-233323**, ***bacc*-233253**, and ***bacc*-233133**; comparison of the optimized geometry of ***bacc*-233253** with the geometry of ***bacc*-A33D53(*tail*-24623f14)** for 1JFF; observed and calculated VCD spectra of baccatin III with IR spectra (13 conformations); and VCD and IR spectra calculated for each conformation of baccatin III. This material is available free of charge via the Internet at http://pubs.acs.org.

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