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Determination of the Absolute Configuration of Ballonigrin Lactone A Using Density Functional Theory Calculations

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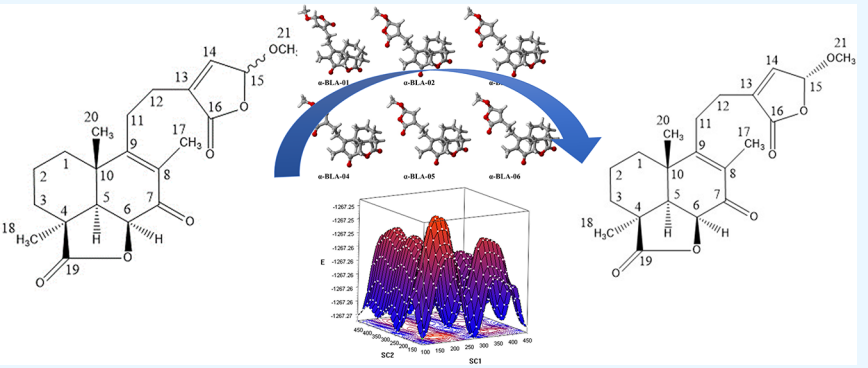

ABSTRACT: We report the determination of the absolute configuration of a diterpenoid, namely, ballonigrin lactone A (BLA), by comparison of the computed optical rotations, $[\alpha]_D$, of its two diastereomers using density functional theory (DFT) calculations to the experimental $[\alpha]_D$ value of +22.4. One of the diastereomers having configurations 4S, 5R, 6S, 10S, 15S was named "α-BLA," and the other one with configuration 4S, 5R, 6S, 10S, 15R was called "β-BLA". Six conformers for each diastereomer (α-BLA and β-BLA) of BLA were identified through their conformational analysis. $[\alpha]_D$ values of these six conformations for each diastereomer were calculated using DFT at the mPW1PW91/6-311G(d,p)/$SMD_{Chloroform}$ level of theory, leading to the conformationally averaged $[\alpha]_D$ values of −96.8 for α-BLA and +65.1 for β-BLA. Thus, it was found that the experimental $[\alpha]_D$ value of +22.4 was of 4S, 5R, 6S, 10S, 15R, i.e., β-BLA. Experimental and computed nuclear magnetic resonance (NMR) data were also compared, and this comparison was in accordance with the conclusion drawn from the comparison of $[\alpha]_D$ values. Finally, the results were augmented with the calculation of the DP4 analysis, and the probability obtained also endorsed our earlier calculations.

1. INTRODUCTION

The trend of collecting plants and associated products is a very old one and continues to date for various purposes, including medicinal importance, food, and trade.[1] One of the plant genera known for these potential applications is the genus *Ballota*, due to which there is a remarkable interest among natural product scientists toward exploring many of its species.[2−6] To date, this genus is known to have 33 species,[7] of which the majority are located in the Mediterranean region. Two of the species from the list, i.e., *Ballota limbata* and *Ballota aucheri*, are found in Pakistan.[8]

One of the potential applications of various *Ballota* species is their therapeutic use.[9] *Ballota nigra*, for instance, is known for its expectorant and antiemetic effects.[10] *Ballota hispanica* has been reported as a sedative and an antidiabetic plant.[5] *Ballota limbata*, locally known as "Awan Buti" in Pakistan, has previously shown potential for the treatment of gum diseases in children and for curing wounds.[11] An important class of bioactive compounds present in *Ballota limbata* consists of diterpenoids,[3,8] which have potential applications in inhibition of lipoxygenase. This enzyme has a role in the biosynthesis of chemical mediators involved in various ailments such as tumor angiogenesis[12] and inflammation.[13]

Ballonigrin lactone A (BLA) is a diterpenoid that was first extracted by Ahmad and Farooq[14] as a sticky solid from one of the fractions obtained from the roots of *Ballota limbata*. High-resolution electron impact mass spectrometry was used to infer the molecular formula of ballonigrin lactone A, i.e., $C_{21}H_{26}O_6$, and the reported structural formula is shown in Figure 1. The experimental data included NMR and $[\alpha]_D$ values for BLA. However, the stereochemistry of carbon number 15 has been

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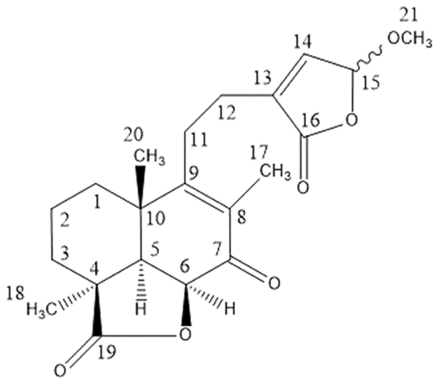

Figure 1. Structure of ballonigrin lactone A.

ambiguous to date. Recent implications of *ab initio* and density functional theory to the calculations of optical rotation and NMR have greatly facilitated the determination of the absolute configuration of natural products with greater authenticity.[15−23]

A common practice in drug development is that many lead compounds are synthesized as a result of inspiration from natural compounds for which the term "Discovery Oriented Synthesis" is used.[24,25] Consequently, many analogues of natural compounds have been synthesized and approved as effective synthetic drugs.[26] Because of the foregoing discussion, it is crucial to unveil the structure of natural compounds for the appraisal of their physiochemical properties and, subsequently, conformable progress in the process of drug development.[27] Additionally, keeping in view the chirality of most natural products and the importance of stereochemistry in drug design, familiarity with the absolute stereochemistry of compounds is of utmost significance, without which the structure would never be completely known. For that purpose, we have selected BLA to gain a computational insight into its stereochemistry at C-15 to confirm its absolute configuration owing to the medicinal importance of this species.

2. COMPUTATIONAL METHODS

All of the calculations in the present study have been performed using Gaussian 16 rev. B.01.[28] The mPW1PW91 hybrid functional[29] was employed, and the 6-311G(d,p) basis set of triple-ζ quality from Pople's group was used. This functional and basis set combination has been shown to perform better than many others for chiroptical property calculations.[30] To include the dispersion effects, Grimme's empirical D3 correction was added along with Becke−Johnson damping (D3BJ). The optimized structures were confirmed as minima due to the absence of any imaginary frequency. The solvent effects of chloroform were added using the polarizable continuum model (PCM) through the integral equation formalism variant with the SMD parameter set established by Cramer and Truhlar.[31]

3. RESULTS AND DISCUSSION

3.1. Conformational Analysis. BLA is conformationally flexible and is capable of acquiring assorted conformations when present in the form of solution at room temperature.[32] Thus, its conformational analysis is the main prerequisite for the computation of its $[\alpha]_D$ because all of the possible conformations need to be considered for the study of flexible compounds.[33] Thus, the relaxed potential energy scans for both α-BLA (4S, 5R, 6S, 10S, 15S) and β-BLA (4S, 5R, 6S, 10S, 15R) were carried out at the mPW1PW91/6-311G(d,p) level of theory. The single rotatable bonds (C11−C12 and C12−C13) were selected in these scans, which were rotated by 15° for a total of 24 steps (Figure 2).

As a result, we ended up with various possible conformers along with their energies. Subsequently, minimum energy structures were selected and optimized at the mPW1PW91/6-311G(d,p)/$SMD_{Chloroform}$ level of theory without any geometry constraints. The optimized structures were then cross-matched to discard any duplicate structures. Finally, six low-energy conformers were obtained for each α-BLA (Figure 3) and β-BLA (Figure 4). These optimized low-energy conformers have been used in all of the subsequent property calculations, including the optical rotation and NMR chemical shifts.

3.2. Optical Rotation Calculation. The optical rotation of all of the low energy-optimized conformers of α-BLA and β-BLA was calculated at the mPW1PW91/6-311G(d,p)/$SMD_{Chloroform}$ level of theory. The computed optical rotations of all of the conformers along with their individual energies have been given in Tables SI-1 and SI-2 in the Supporting Information. The computed optical rotation value of the β-

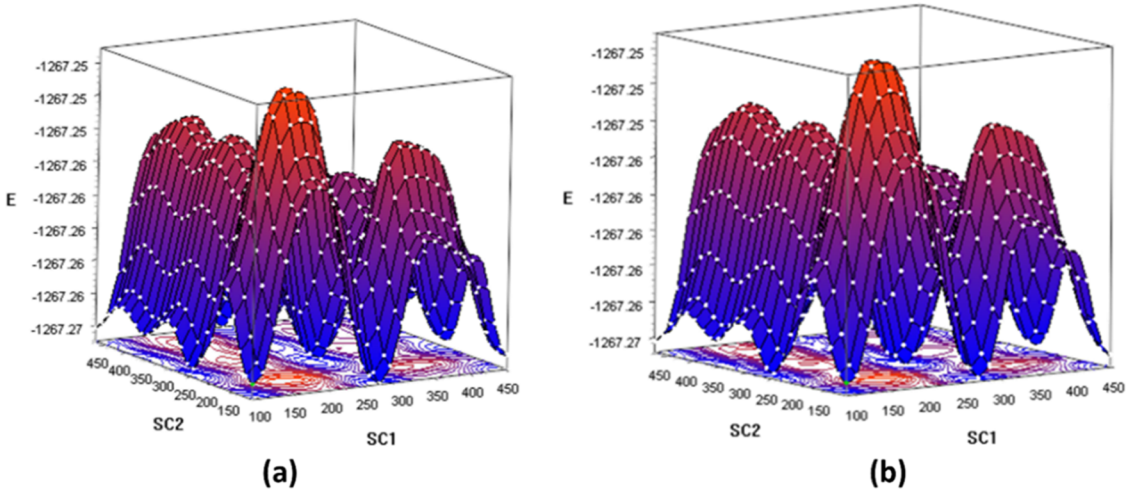

Figure 2. PES for ballonigrin lactone A. (a) α-BLA, (b) β-BLA.

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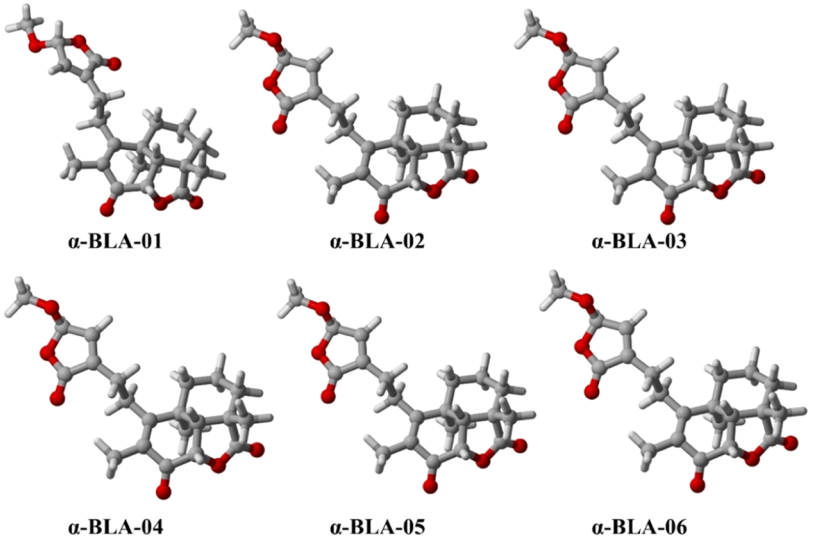

Figure 3. Six optimized conformers of α-BLA at the mPW1PW91/6-311G(d,p)/$SMD_{Chloroform}$ level of theory.

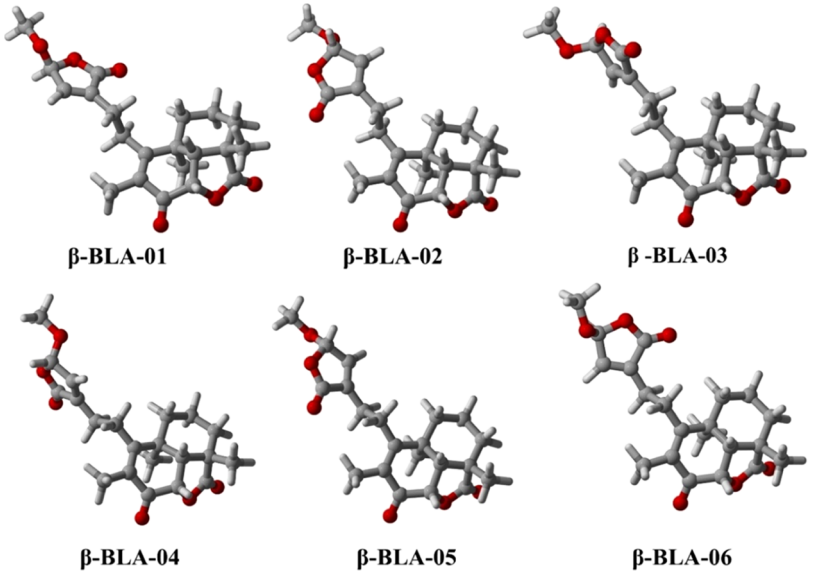

Figure 4. Six optimized conformers of β-BLA at the mPW1PW91/6-311G(d,p)/$SMD_{Chloroform}$ level of theory.

BLA and the experimental $[\alpha]_D$ value both had a positive sign (Table 1). Hence, it was deduced that the experimental $[\alpha]_D$ value was of the diastereomer initially called β-BLA, i.e., 4S, 5R, 6S, 10S, 15R (Figure 5). There have been some reports in the past about not using the optical rotations alone for the assignment of absolute configurations and use it in conjunction with other techniques like ECD, VCD, etc.[34,35] In our case, due to losing the only isolated sample during handling, ECD could not be performed. To overcome this concern, DP4 analysis has been performed with the NMR data, which also augmented the inferences obtained from the optical rotation calculation. DP4 analysis is a very powerful tool to correctly assign the NMR data of diastereomers when only one set of

Table 1. Comparison of the Experimental and Boltzmann Averaged Computed Optical Rotation Values at the mPW1PW91/6-311G(d,p)/$SMD_{Chloroform}$ Level of Theory (deg mL g^{-1} dm^{-1})

compound	optical rotation ($[\alpha]_D$)
experimental $[\alpha]_D$	+22.4
calculated $[\alpha]_D$ for α-BLA	−96.8
calculated $[\alpha]_D$ for β-BLA	+65.1

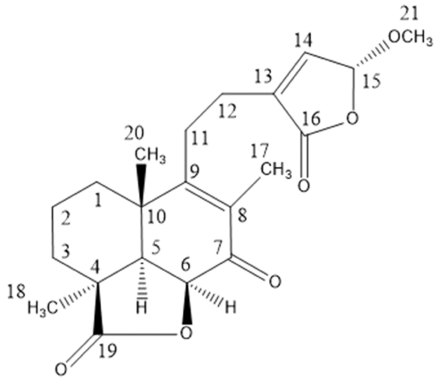

Figure 5. Structure of ballonigrin lactone A (β-BLA).

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experimental data is available and has been used in the past to assign absolute configurations along with the optical rotation calculation when other spectroscopic methods have not been available.[36]

3.3. Nuclear Magnetic Resonance (NMR) Calculation. NMR spectroscopy is undoubtedly an unparalleled technique for the depiction of the structural traits of a compound such as connectivity information, functional groups' details, neighboring atoms, spatial interactions, etc. Since the last decade, quantum chemistry has deeply penetrated its roots in the structure elucidation processes. Many methods have been developed that allow chemists to differentiate among stereoisomers even when the experimental data for only one of them is available. To predict the NMR chemical shifts for supple molecules, their energy minimum conformers need to be identified on the PES, which we had already located. The ^{13}C and ^{1}H NMR computations were carried out at the mPW1PW91/6-311G(d,p)/$SMD_{Chloroform}$ level of theory. Magnetic shielding tensors were obtained that were then converted to chemical shifts using the multistandard referencing approach by Sarotti and Pellegrinet.[37] According to this approach, sp^3 carbons are referenced using methanol, whereas sp and sp^2 carbons are referenced using benzene.

Finally, Boltzmann averaging of the chemical shifts of all conformers was done according to their relative energies, and then, the experimental and computed data sets of NMR were compared. The experimental NMR data appeared to be in a better settlement with the computed data of β-BLA as compared to α-BLA. The mean absolute errors for ^{1}H NMR were 0.564 ppm for α-BLA (Tables SI-3 and SI-6) and 0.555 ppm for β-BLA (Table 2). The lower value of MAE of ^{1}H NMR in the case of β-BLA implied that the experimental NMR data belonged to a compound with $-OCH_3$ below the plane at carbon number 15. The low value of MAE is an indication of good conformational analysis because the chemical shifts in ^{13}C NMR and ^{1}H NMR are very fine-tuned with regard to their chemical environments. Hence, a decent conformational ensemble is vital for its correct representation. The comparison of the ^{13}C NMR chemical shifts has been given in Table 3 for the β-BLA and in Table SI-4 for α-BLA. The mean absolute error for ^{13}C NMR was 2.74 ppm for α-BLA (Tables SI-4 and SI-6) and 2.60 ppm for β-BLA (Tables 3 and SI-7). Mean absolute errors (MAEs) were calculated by adding the absolute differences between the actual and calculated values of each observation over the entire array and then dividing the sum obtained by the total number of observations.

Table 2. Comparison of the Experimental and Boltzmann Averaged Computed ^{1}H NMR Data for β-BLA

carbon type	carbon no.	^{1}H NMR (experimental), δ (ppm)	^{1}H NMR (computed), δ (ppm)	$\Delta\delta$ (ppm)
CH_2	1	1.35	1.34	0.01
		1.40	0.96	0.44
CH_2	2	1.75	1.25	0.50
		1.75	1.12	0.63
CH_2	3	1.98	0.83	1.15
		1.98	1.73	0.25
C	4			
CH	5	2.90	1.48	1.42
CH	6	4.86	4.13	0.73
CH_2	11	2.46	2.29	0.17
		2.46	1.90	0.56
CH_2	12	2.50	1.94	0.56
		2.50	1.75	0.75
CH	14	6.72	6.60	0.12
CH	15	5.73	5.18	0.55
CH	17	1.88	1.44	0.44
CH	18	1.40	0.69	0.71
CH	20	1.12	0.49	0.63
OCH_3	OCH_3	3.57	3.19	0.38

Table 3. Comparison of the Experimental and Boltzmann Averaged Computed ^{13}C NMR Data for β-BLA

carbon type	carbon no.	^{13}C NMR (experimental), δ (ppm)	^{13}C NMR (computed), δ (ppm)	$\Delta\delta$ (ppm)
CH_2	1	28.1	30.75	−2.65
CH_2	2	21.9	18.96	2.94
CH_2	3	27.6	28.39	−0.79
C	4	42.2	41.95	0.25
CH	5	49.3	51.32	−2.02
CH	6	78.8	75.02	3.78
C	7	194.1	196.72	−2.62
C	8	135.7	136.85	−1.15
C	9	165.3	172.12	−6.82
C	10	39.1	38.65	0.45
CH_2	11	29.9	27.38	2.52
CH_2	12	24.6	26.89	−2.29
C	13	139.1	138.85	0.25
CH	14	141.3	144.82	−3.52
CH	15	100.9	103.44	−2.54
C	16	170.6	172.63	−2.03
CH	17	17.4	11.74	5.66
CH	18	26.7	24.58	2.12
C	19	176.4	182.00	−5.60
CH	20	22.4	26.26	−3.86
OCH_3	OCH_3	56.2	56.92	−0.72

3.4. DP4 Analysis. DP4 analysis is one of the widely used tools to confirm one of the two diastereoisomers when only one experimental data set is available.[38] DP4 is a robust system for the processing and assignment of data obtained from ^{13}C and ^{1}H NMR. This analysis has represented a leap forward in the practice of elucidating structures. It has assisted in the analyses of data sets belonging to bulky molecules and even revision of some wrongly assigned structures.[19,39−46] To further validate the results after optical rotation and NMR chemical shift calculations, we fed the experimental and computed data sets of NMR into the DP4 applet by Goodman et al., and the results of DP4 analysis showed that our interpretation of the NMR data and the optical rotation calculation was correct. The DP4 analysis results have been presented in Table 4.

Table 4. Results of the DP4 Analysis for BLA

results of DP4 using both carbon and proton data:	α-BLA: 0.6%	β-BLA: 99.4%
results of DP4 using carbon data only:	α-BLA: 3.6%	β-BLA: 96.4%
results of DP4 using proton data only:	α-BLA: 14.8%	β-BLA: 85.2%

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4. CONCLUSIONS

In this study, we aimed to uncover the absolute structure of a diterpenoid, ballonigrin lactone A (BLA), whose antibacterial activity has been reported previously and could also be the focus of attention for scientists in the future. Regrettably, the absolute structure of this diterpenoid was not known and it could be one of the possible hurdles in exploring its beneficial traits. Therefore, we decided to play our role in the community of research by taking this small step forward. BLA is a flexible molecule due to which its conformational analysis has been done by running relaxed PESs. The different conformers that were obtained from the scans were optimized at the mPW1PW91/6-311G(d,p)/$SMD_{Chloroform}$ level of theory. The resultant low-energy conformers for both possibilities of the substituent at C-15 have been subjected to NMR and optical rotation calculations at the same level of theory, followed by Boltzmann averaging of the results. The experimental optical rotation value of +22.4 correlated well with the structure having OCH_3 at C-15 in β-configuration. The calculated NMR chemical shifts also correlated well with the experimental ones, showing the mean absolute error of only 0.42 ppm for ^1H NMR. The results were further augmented by DP4 analysis, which also confirmed the true structure to be as shown in Figure 5.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03858.

Energies and optical rotation values of individual low-energy conformers; comparison of the experimental and Boltzmann averaged computed NMR data for α-BLA; and the calculation of optical rotation values at the CAM-B3LYP/aug-cc-pVDZ level of theory (PDF)

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Author Contributions

‖M.N. and S.A. have equal contributions.

Notes

The authors declare no competing financial interest.

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