Vibrational analysis of deoxy-andrographolide using MM/QM methods

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Abstract. Bioactive natural products have an enormous economic importance as speciality chemicals. Extracts of the shrub *Andrographis paniculata* are widely used as herbal medicines in many countries. Diterpenoid lactones isolated from these extracts have been the subject of extensive investigation, and one of its constituent diterpenoid is deoxy-andrographolide. A complete normal coordinate analysis was performed for deoxy-andrographolide in terms of the calculation by using Classical mechanics and Quantum mechanical methods. The study of vibrational spectra helps in understanding the dynamical behaviour and hence a lot of information can be derived about the conformation. The geometrical parameters and the vibrational spectra have been discussed in detail.

Keywords: FT-IR spectra, lactones, normal mode analysis, MM, Hartree–Fock

1. Introduction

Natural products offer unmatched structural variety, especially as new environmental niches are explored, and their usefulness can be further extended by engineering the proteins that produce them and using them to probe biological pathways [1]. Rediscovery of the connection between plants and health is responsible for launching a new generation of botanical therapeutics that include plant derived therapeutics. Natural products evolved to perform a function that is achieved by binding to proteins. Therefore, natural product should be able to penetrate the biological barrier and make their ways to certain cells or organs in which they will exert their effect. Thus, natural product is already validated to reach and bind specific proteins. The similarity in protein domain structure can be exploited to develop inhibitors of enzymes with different functions based on the guiding structure of a natural product.

Natural compounds contains a range of complex organic chemicals that may have synergistic effect on various physiological process in normal and disease state by interacting with multiple metabolic pathways [2]. *Andrographis paniculata*, a medicinal herb is reported to have antidiabetic [3] and antithrombotic [4] effects. It belongs to the family Acanthaceae and is a rich source of many bioactive

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compounds. Its antioxidant and antiinflammatory activity has already been reported [5]. Major active component of this plant andrographolide has shown to possess pharmacological properties which encompass antihepatotoxic [6] and antiviral effects [7]. It has recently been reported that andrographolide inhibits NF- κ B binding to DNA and thus reducing the expression of proinflammatory proteins such as cyclooxygenase-2 (COX-2) [8].

The present work on the diterpenoid “deoxy-andrographolide” is in continuation to our earlier work on andrographolide [9] and neo-andrographolide [10]. Since this constituent of *Andrographis paniculata* (AP) has been identified as scaffold for the generation of combinatorial libraries using solid phase methods [11], it was decided to study its molecular structure and vibrational spectra in detail. This compound has also been widely used for the treatment of calcium channel blocking activity, platelet activating factor (PAF) antagonistic activity, anti-inflammatory activity, anti-cancer activity apart from many other like analgesic, antipyretic, anti ulcerogenic effects and hence it has aroused the interest of researchers to probe in detail its structure and dynamics [12–14].

To the best of our knowledge no study on the normal coordinate analysis on deoxy-andrographolide has been reported so far in the literature apart from few spectroscopic studies [15,16]. As a part of our ongoing research work [17,18] on vibrational analysis in a variety of macromolecules, here in the present communication, the experimental FT-IR frequencies of the title molecule deoxy-andrographolide are compared with theoretical frequencies obtained by normal coordinate analysis using two different methods: a classical mechanics method and the Ab initio Hartree-Fock method. Thus the evaluation of the advantages of both the methods is useful for obtaining a reliable assignment of the vibrational spectra. The purpose of this study assumes importance because of our earlier work [9] on the major constituent of AP i.e. andrographolide and hence a comparative study between the two diterpenes can be carried out.

2. Computational methods

2.1. Classical mechanics calculations

The normal coordinate analysis is based on the Wilson GF matrix method [19–21] employing Urey Bradley force field [22] to evaluate normal modes. The Cartesian coordinates were calculated from X-ray crystallographic data obtained by Rajnikant et al. [23]. The title compound Deoxy-andrographolide, a diterpenoid, crystallizes in the monoclinic space group P_{21} . The six membered rings are trans-fused and both are in the chair conformation. The furan ring is planar. Molecules are held together by C–H...O and O–H...O hydrogen bonds. Basak et al. [24,25] have shown that andrographolide could be considered as an important precursor structure that might require further structure modification for enhancement of its enzyme inactivation property. The modification of hydroxyl groups in *Andrographis paniculata* could lead to a better understanding of its inhibitory action. The actual mechanism by which these diterpenes exert their inhibitory action against convertases is not currently understood and that the molecule contains a highly reactive five membered lactone ring.

2.2. Quantum mechanical calculations

The entire calculations involving Hartree–Fock (HF) level were performed on the Pentium IV/2.9 GHz personal computer using Gaussian 03W program package [26], invoking gradient geometry optimization

[27]. Initial geometry generated from standard geometrical parameters was minimized without any constraint in the potential energy surface at Hartree–Fock level, adopting the standard 3–21 basis set. In order to obtain a more complete description of the molecular motion the vibrational frequency calculations were carried out at HF level. Then vibrationally averaged nuclear positions of Deoxy-andrographolide were used for harmonic vibrational frequency calculation resulting in IR frequencies.

By combining the result of GaussView program [28] with symmetry considerations, vibrational frequency assignments were made with a high degree of accuracy. The defined coordinates form a complete set and matches well with the motions observed using the GaussView program.

3. Experimental

The FTIR spectra of Deoxy-andrographolide have been recorded in CsI on a Perkin Elmer 1800 spectrophotometer. Spectroscopic preparation of sample were carried out under an atmosphere of prepurified nitrogen. Deoxy-andrographolide was isolated from fresh plant material of *Andrographis paniculata* to the procedure reported in literature [29]. The compound was identified by comparison with its IR, MS, NMR data with that of reported in literature [29,30].

Isolation: plant material (*Andrographis paniculata*, 1 kg) was powdered and percolated with ethanol (3×5 l). The ethanolic extract was concentrated in vacuo at 50°C. This was diluted with water (750 ml) and fractionated to hexane (9 g), chloroform (10.6 g), butanol (30 g) and aqueous fractions (25 g). The butanol fraction (10 g) was chromatographed over a column of silica gel (300 g) in chloroform and eluted with chloroform, chloroform: ethylacetate, ethylacetate, ethylacetate: methanol, chloroform: ethylacetate eluate was further purified by rechromatography and crystallization with chloroform gave deoxyandrographolide (400 mg, 0.04%): m.p. 169°C. Mass spectrum (FAB) m/z 335, 357 ($M^+ + Na$); 1H NMR (400 MHz, $CDCl_3$): 7.10 (brs, 1H, H-14), 4.89 (s, 1H, H-17), 4.78 (s, 2H, H-15), 4.60 (s, 1H, H-17), 4.16 (d, 12, 1H, H-19), 3.48 (t, 6, 1H, H-3), 3.31 (d, 12, 1H, H-19), 2.40–2.50 (m, 2H, H-9 & H-7), 2.00–1.00 (m, 12H, $6 \times CH_2$), 1.21 and 0.70 (2s, 3H each, H-20 & H-18).

4. Result and discussion

4.1. Vibrational investigation

4.1.1. Molecular mechanics calculations

Normal coordinate calculations were performed using the Shimanouchi program [31] which follows the Wilson's G-F matrix method. This method describes the motion in terms of the internal coordinates, which are changes in bond lengths, bond angles and those out of plane bending and dihedral angles. The force constants in terms of these coordinates can be easily visualized and have a physical meaning. We have used a Urey–Bradley force field in our calculations. It incorporates intra unit interactions and interactions due to the neighboring units, in addition to the bonded interactions. It also includes the interactions between non-bonded atoms. Force constants were initially taken from the literature [32] and later modified to give the “best fit” with the observed FT-IR spectra.

The Deoxy-andrographolide has 54 atoms, however to reduce the problem to manageable dimensions CH, CH_2 , CH_3 have been treated as mass points with a mass of 13, 14 and 15 respectively. This does not in any way disturb the accuracy of the results reported here. This is because the frequencies belonging to these can be well designated as group frequencies and many of them being in the higher range do not mix

with other modes. With this approximation the andrographolide problem reduces to 24 atom problem with 66 normal modes of vibrations. The structural data used for Deoxy-andrographolide is reported by Rajnikant et al. [23]. The model molecular structure of deoxy-andrographolide used in normal coordinate analysis is given in Fig 1. The internal coordinates and force constants for deoxy-andrographolide are given in Table 1. The final force constant values for the corresponding modes are given in Table 2.

Fig. 1. Model molecular structure of 14-deoxyandrographolide.

Table 1
Internal co-ordinates and Urey–Bradley force constants (md/Å)

Internal coordinate	Force constant	Internal coordinate	Force constant
1. $\nu(\text{C1}–\text{C2})$	3.300	47. $\Phi(\text{C8}–\text{C9}–\text{C11})$	0.460 (0.420)
2. $\nu(\text{C1}–\text{C10})$	3.300	48. $\Phi(\text{C10}–\text{C9}–\text{C11})$	0.460 (0.420)
3. $\nu(\text{C2}–\text{C3})$	3.300	49. $\Phi(\text{C1}–\text{C10}–\text{C5})$	0.880 (0.850)
4. $\nu(\text{C3}–\text{C4})$	3.300	50. $\Phi(\text{C1}–\text{C10}–\text{C9})$	0.560 (0.700)
5. $\nu(\text{C3}–\text{O21})$	4.800	51. $\Phi(\text{C1}–\text{C10}–\text{C18})$	0.460 (0.400)
6. $\nu(\text{C4}–\text{C5})$	3.300	52. $\Phi(\text{C5}–\text{C10}–\text{C9})$	0.410 (0.400)
7. $\nu(\text{C4}–\text{C19})$	4.100	53. $\Phi(\text{C5}–\text{C10}–\text{C18})$	0.560 (0.500)
8. $\nu(\text{C4}–\text{C20})$	5.130	54. $\Phi(\text{C9}–\text{C10}–\text{C18})$	0.760 (0.700)
9. $\nu(\text{C5}–\text{C6})$	3.200	55. $\Phi(\text{C9}–\text{C11}–\text{C12})$	0.980 (0.980)
10. $\nu(\text{C5}–\text{C10})$	4.300	56. $\Phi(\text{C11}–\text{C12}–\text{C13})$	0.980 (0.900)
11. $\nu(\text{C6}–\text{C7})$	3.200	57. $\Phi(\text{C12}–\text{C13}=\text{C14})$	1.400 (0.750)
12. $\nu(\text{C7}–\text{C8})$	3.200	58. $\Phi(\text{C12}–\text{C13}–\text{C16})$	0.560 (0.560)
13. $\nu(\text{C8}–\text{C9})$	3.200	59. $\Phi(\text{C14}=\text{C13}–\text{C16})$	0.760 (0.590)
14. $\nu(\text{C8}=\text{C17})$	9.410	60. $\Phi(\text{C13}=\text{C14}–\text{C15})$	0.760 (0.590)
15. $\nu(\text{C9}–\text{C10})$	3.200	61. $\Phi(\text{C14}–\text{C15}–\text{C22})$	0.530 (0.520)

Table 1
(Continued)

Internal coordinate	Force constant	Internal coordinate	Force constant
16. $\nu(\text{C9-C11})$	5.650	62. $\Phi(\text{C13-C16-O22})$	0.530 (0.520)
17. $\nu(\text{C10-C18})$	4.748	63. $\Phi(\text{C13-C16=O23})$	1.550 (0.760)
18. $\nu(\text{C11-C12})$	4.600	64. $\Phi(\text{C22-C16=O23})$	0.860 (0.560)
19. $\nu(\text{C12-C13})$	5.390	65. $\Phi(\text{C4-C19-O24})$	0.580 (0.650)
20. $\nu(\text{C13=C14})$	6.890	66. $\Phi(\text{C15-O22-C16})$	1.200 (0.760)
21. $\nu(\text{C13-C16})$	4.645	67. $\omega(\text{C17=C18})$	0.215
22. $\nu(\text{C14-C15})$	4.645	68. $\omega(\text{C18-C10})$	0.215
23. $\nu(\text{C15-O22})$	5.000	69. $\omega(\text{C20-C4})$	0.225
24. $\nu(\text{C16-O22})$	5.300	70. $\omega(\text{O21-C3})$	0.225
25. $\nu(\text{C16=O23})$	8.352	71. $\omega(\text{O23=C16})$	0.225
26. $\nu(\text{C19=O24})$	6.410	72. $\tau(\text{C1-C2})$	0.025
27. $\Phi(\text{C2-C1-C10})$	0.880 (0.850)	73. $\tau(\text{C1-C10})$	0.025
28. $\Phi(\text{C1-C2-C3})$	0.880 (0.850)	74. $\tau(\text{C2-C3})$	0.025
29. $\Phi(\text{C2-C3-C4})$	0.880 (0.850)	75. $\tau(\text{C3-C4})$	0.025
30. $\Phi(\text{C2-C3-C21})$	0.810 (0.760)	76. $\tau(\text{C4-C5})$	0.025
31. $\Phi(\text{C4-C3-O21})$	0.810 (0.760)	77. $\tau(\text{C4-C19})$	0.025
32. $\Phi(\text{C3-C4-C5})$	0.880 (0.850)	78. $\tau(\text{C5-C6})$	0.025
33. $\Phi(\text{C3-C4-C19})$	0.460 (0.400)	79. $\tau(\text{C5-C10})$	0.022
34. $\Phi(\text{C3-C4-C20})$	0.460 (0.450)	80. $\tau(\text{C6-C7})$	0.025
35. $\Phi(\text{C5-C4-C19})$	0.460 (0.400)	81. $\tau(\text{C7-C8})$	0.025
36. $\Phi(\text{C5-C4-C20})$	0.460 (0.450)	82. $\tau(\text{C8-C9})$	0.025
37. $\Phi(\text{C19-C4-C20})$	0.660 (0.560)	83. $\tau(\text{C9-C10})$	0.025
38. $\Phi(\text{C4-C5-C6})$	0.560 (0.700)	84. $\tau(\text{C9-C11})$	0.025
39. $\Phi(\text{C4-C5-C10})$	0.880 (0.850)	85. $\tau(\text{C11-C12})$	0.025
40. $\Phi(\text{C6-C5-C10})$	0.410 (0.400)	86. $\tau(\text{C12-C13})$	0.025
41. $\Phi(\text{C5-C6-C7})$	0.410 (0.400)	87. $\tau(\text{C13-C14})$	0.022
42. $\Phi(\text{C6-C7-C8})$	0.410 (0.400)	88. $\tau(\text{C13-C16})$	0.018
43. $\Phi(\text{C7-C8-C9})$	0.410 (0.400)	89. $\tau(\text{C14-C15})$	0.018
44. $\Phi(\text{C7-C8=C17})$	0.880 (0.940)	90. $\tau(\text{C15-O22})$	0.017
45. $\Phi(\text{C9-C8=C17})$	0.880 (0.940)	91. $\tau(\text{C16-C22})$	0.018
46. $\Phi(\text{C8-C9=C10})$	0.410 (0.400)		

$\nu(\text{X-Y})$ represents the stretching between the atoms X and Y etc.

$\Phi(\text{X-Y-Z})$ represents the in plane bending between the atoms X, Y and Z.

$\omega(\text{X-Y})$ represents the out of plane bending (wagging) between the atoms X and Y.

$\tau(\text{X-Y})$ represents the torsion of the atoms X and Y.

The number in parenthesis represents the non-bonded value of the atoms X, Y and Z.

The frequencies along with the potential energy distribution are given in Table 3. The FT-IR spectra of Deoxy-andrographolide is given in Fig 2. The observed frequencies agree with the calculated ones within 5 cm^{-1} . In the assignment of the normal modes only the dominant potential energy distributions are given. Identification with the experimental data has been made on the basis of potential energy distribution, line profile, relative intensities, energies and the presence/absence of a given mode in similar molecules.

Although a number of spectroscopic studies [32,33] have been performed on andrographis paniculata but presumably, the data for the normal mode analysis on deoxy-andrographolide is being reported for

Table 2
Force constant values for corresponding modes

S. no	Modes	Force constant values
1	v(C–C) R1	3.3000
2	v(C–C) R2	3.2000
3	v(C–C) R3	4.6450
4	v(C=C) R3	6.8900
5	v(C–C) cm R1&R2	4.3000
6	v(C–O) adj R1	4.8000
7	v(C–C) adj R1 link O	4.1000
8	v(C–C) adj R1	5.1300
9	v(C=C) adj R2	9.4100
10	v(C–C) adj R2	5.6500
11	v(C–C) adj cm R1&R2	4.7480
12	v(C–C) BB	4.6000
13	v(C–C) adj R3	5.3900
14	v(C–O) R3	5.0000
15	v(C–O) R3 adj (=O)	5.3000
16	v(C=O) adj R3	8.3520
17	v(C–O) link R1	6.4100
18	a(C–C–C) R1	0.8800
19	a(C–C–C) R2	0.4100
20	a(C=C–C) R3	0.7600
21	a(C–C–C) adj R1 ln O	0.4600
22	a(C–C–C) o/s R2	0.6600
23	a(O–C–C) cm R1&R2	0.5600
24	a(C–C=C) adj R2	0.8800
25	a(C–C–C) adj R2 ln BB	0.4600
26	a(C–C–C) adj R1 ter	0.4601
27	a(C–C–C) cm R1&R2	0.5600
28	a(C–C–C) adj R2	0.7600
29	a(C–C–C) R2 ln BB	0.9800
30	a(C–C–C) BB	0.9800
31	a(C–C=C) adj R3	1.4000
32	a(C–C–C) adj R3	0.5600
33	a(C–C–O) R3	0.5300
34	a(C–C=O) adj R3	1.5500
35	a(O–C=O) adj R3	0.8600
36	a(C–C–O) adj R1 ln O	0.5800
37	a(C–O–C) R3	1.2000
38	a(C–C–O) adj R1	0.8100
39	w(C–C) R2	0.2150
40	w(C–C) cm R1&R2	0.2150
41	w(C–C) R1	0.2250
42	w(C–C) R1	0.2250
43	w(O–C) R3	0.2250
44	t(C–C) R1	0.0250
45	t(C–C) adj R1	0.0250

Table 2
(Continued)

S. no	Modes	Force constant values
46	t(C–C) R2	0.0250
47	t(C–C) adj R2	0.0250
48	t(c–C) BB	0.0250
49	t(C–C) adj R3	0.0250
50	t(C=C) R3	0.0220
51	t(C–C) R3	0.0180
52	t(C–O) R3	0.0170
53	t(C=O) adj R3	0.0180
54	t(C–C) cm R1&R2	0.0220

Abbreviations used in the table have the following meanings: R – ring; adj – adjacent to; BB – back bone; cm – common to; ln – linking; o/s – outside.

Table 3

Calculated freq. (cm ⁻¹)	Observed freq. (cm ⁻¹)	Assignment (% potential energy distribution)
1754	1754	$\nu(\text{C=O})$ adj R3 [51] + $\nu(\text{C–C})$ R3 [41] + $\nu(\text{C–C})$ adj R3 [9]
1697	1700	$\nu(\text{C=C})$ adj R2 [96] + $\nu(\text{C–C})$ R2 [11]
1632	1636	$\nu(\text{C=C})$ R3 [63] + $\nu(\text{C–C})$ adj R3 [28] + $\nu(\text{C–C})$ R3 [5]
1594	1595	$\nu(\text{C–C})$ adj R2 [44] + $\nu(\text{C–C})$ R1 [34] + $\nu(\text{C–C})$ BB [6]
1463	1453	$\nu(\text{C–C})$ R1 [32] + $\nu(\text{C–O})$ adj R1 [15] + $\Phi(\text{C–C–O})$ adj R1 link O [14] + $\nu(\text{C–C})$ adj R1 [8] + $\nu(\text{C–O})$ link R1 [8] + $\Phi(\text{C–C–O})$ adj R1 [7] + $\Phi(\text{C–C–C})$ adj R1 ter [6]
1385	1389	$\nu(\text{C–C})$ cmn R1&R2 [48] + $\nu(\text{C–C})$ adj cmn R1&R2 [17] + $\nu(\text{C–C})$ R1 [12] + $\Phi(\text{C–C–C})$ R1 [12] + $\nu(\text{C–O})$ adj R1 [12] + $\nu(\text{C–C})$ R2 [5] + $\Phi(\text{C–C–C})$ adj R2 [8]
1351	1351	$\nu(\text{C–C})$ adj R1 [37] + $\nu(\text{C–O})$ adj R1 [24] + $\nu(\text{C–C})$ R1 [12] + $\Phi(\text{C–C–C})$ R1 [6] + $\nu(\text{C–C})$ adj R1 link O [6]
1300	1297	$\nu(\text{C–C})$ R1 [53] + $\nu(\text{C–O})$ link R1 [15] + $\nu(\text{C–C})$ adj R1 link O [15] + $\nu(\text{C–O})$ adj R1 [7] + $\nu(\text{C–C})$ R2 [6]
1278	1270	$\nu(\text{C–C})$ R1 [53] + $\nu(\text{C–C})$ adj cmn R1&R2 [14] + $\nu(\text{C–C})$ BB [13] $\nu(\text{C–C})$ adj R21 [11] + $\nu(\text{C–C})$ adj R1 [10] + $\nu(\text{C–C})$ cmn R1 & R2 [7] + $\nu(\text{C–C})$ R2 [5]
1207	1207	$\nu(\text{C–C})$ R3 [62] + $\nu(\text{C–C})$ adj R3 [10] + $\Phi(\text{C–C=C})$ adj R3 [5] + $\nu(\text{C–C})$ R2 [5]
1149	1158	$\nu(\text{C–O})$ link R1 [38] + $\nu(\text{C–C})$ R1 [34] + $\nu(\text{C–C})$ R2 [16] + $\nu(\text{C–C})$ adj R1 link O [6]
1139	1130	$\nu(\text{C–C})$ BB [50] + $\nu(\text{C–C})$ R1 [31] + $\nu(\text{C–C})$ R2 [8]
1082	1090	$\nu(\text{C–C})$ R2 [45] + $\nu(\text{C–O})$ link R1 [14] + $\nu(\text{C–C})$ R2 [14] + $\Phi(\text{C–C–C})$ R1 [13] + $\nu(\text{C–C})$ adj R1 link O [9]
1047	1038	$\nu(\text{C–O})$ R3 [32] + $\nu(\text{C=C})$ R3 [13] + $\nu(\text{C–C})$ adj R3 [12] + $\Phi(\text{C–C–C})$ BB [6] + $\Phi(\text{C–C–C})$ R2 ln BB [5]
956	952	$\nu(\text{C–C})$ R1 [40] + $\nu(\text{C–O})$ adj R1 [16] + $\nu(\text{C–C})$ R2 [16] + $\nu(\text{C–C})$ adj R2 [6]
913	903	$\nu(\text{C–C})$ R3 [22] + $\nu(\text{C–O})$ R3 [18] + $\Phi(\text{C–C–C})$ R2 ln BB [8] + $\Phi(\text{C–C–C})$ BB [6] + $\nu(\text{C–O})$ R3 adj (=O) [5]
852	850	$\tau(\text{C=O})$ adj R3 [52] + $\tau(\text{C–O})$ R3 [30] + $\tau(\text{C–C})$ R3 [6] + $\tau(\text{C–C})$ adj R3 [6]
756	757	$\Phi(\text{C–C–C})$ R1 [21] + $\nu(\text{C–C})$ R1 [15] + $\nu(\text{C–C})$ R2 [14] + $\nu(\text{C–O})$ adj R1 [7]

Table 3
(Continued)

Calculated freq. (cm ⁻¹)	Observed freq. (cm ⁻¹)	Assignment (% potential energy distribution)
714	713	$\Phi(\text{C-C-C})$ R2 [20] + $\nu(\text{C-C})$ R1 [10] + $\Phi(\text{C-C-C})$ adj R2 ln BB [8] + $\Phi(\text{C-C-C})$ R1 [8] + $\Phi(\text{C-C-C})$ R2 ln BB [7] + $\Phi(\text{C-C=C})$ adj R2 [7] + $\Phi(\text{C=C-C})$ R3 [6] + $\Phi(\text{C-C-O})$ R3 [5] + $\nu(\text{C-C})$ R2 [5]
639	642	$\Phi(\text{C-C=C})$ adj R2 [29] + $\nu(\text{C-C})$ R2 [20] + $\Phi(\text{C-C-C})$ R1 [7] + $\Phi(\text{C-C-C})$ R2 [6] + $\Phi(\text{O-C-C})$ cmn R1&R2 [5]
576	566	$\Phi(\text{C-C-C})$ R2 [17] + $\Phi(\text{C-C=C})$ adj R2 [12] + $\Phi(\text{C-C-C})$ R1 [12] + $\nu(\text{C-C})$ R1 [8] + $\nu(\text{C-C})$ R2 [7] + $\Phi(\text{C-C-O})$ adj R1 ln O [6] + $\Phi(\text{C-C-C})$ adj R1 ln O [5]

Abbreviations used in the table have following meanings: R – ring; adj – adjacent to; ln – linking; cmn – common to; BB – back bone.

the first time and this makes it imperative to discuss all the vibrational modes with significant potential energy distribution and hence their relation with the conformation of deoxy-andrographolide (Fig. 1). All the significant vibrational modes involving the prime sites are discussed as follows.

Rings R1, R2 and R3. Both the andrographolide and deoxy-andrographolide are diterpenes. Both have identical structure except ring R3. The andrographolide has a hydroxyl group at C14 and the C16=O23 carbonyl group is in conjugation with C12–C13, whereas in deoxy-andrographolide, hydroxyl group at C14 is absent. In place of hydroxyl group a double bond in the ring R3 at C13=C14 is present which is in conjugation with the C16=O23 carbonyl group. This is supported by a low potential energy distribution value (C12–C13) 28% in deoxy-andrographolide and high P.E.D. value 47% in andrographolide. The planarity of ring R3 in deoxy-andrographolide may be because of this difference which is also evidenced by X-ray studies.

The C=O stretching mode at 1728 cm⁻¹ of andrographolide is comparable to 1754 cm⁻¹ in deoxy-andrographolide. The (C16–O23) 51% in deoxy-andrographolide can be compared to (C16–O23) 71% in andrographolide. This value can also be compared with C=O stretch mode in gamma-butyrolactone [34] in which it appears at 1772 cm⁻¹. This value is very typical for similar modes in lactones and identical fragments [32].

The five membered ring R3 has a planar orientation which was also illustrated by low value of torsion angles (C10–C9–C11–C12), (C9–C11–C12–C13) and (C11–C12–C13–C16) as shown by X-ray studies also. A slightly high P.E.D. i.e. 49% (C9–C11) in deoxy-andrographolide as compared to 44% in andrographolide may be because of this reason.

As suggested by the crystallographic study and along with our normal mode calculations it can be said that the ring R3 remains in a planar form and R1, R2 in chair form. The P.E.D. data further suggests that C attached to heteroatom i.e., O has high P.E.D. value (C15–O22) 32%. The low value of (C3–O21) 15% as compared to (C16–O23) 51% is suggestive of the close proximity of (C16–O23) by ring junction (C12–C11) 50% and rings R1 and R2. For the vibrational band at 639 cm⁻¹ the normal mode calculation yields a P.E.D. with $\Phi(\text{C-C=C})$ 29% whereas the deformation $\Phi(\text{C-C-C})$ is only 7%.

4.2. Quantum mechanical Hartree–Fock calculations

4.2.1. Molecular geometry

The optimized structure parameters of Deoxy-andrographolide calculated by ab initio HF level with 3–21 basis set are listed in Table 4 in accordance with the atom numbering scheme given in Fig. 1. For

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Fig. 2. FT-IR spectra of Deoxyandrographolide (4000–700) cm^{-1} .

example, the optimized bond lengths of C–C in R1 ring falls in the range from 1.512 Å to 1.564 Å for HF, which is in good agreement with those of experimental bond length for deoxy andrographolide (1.519–1.590 Å). The optimized bond length C13–C14 for HF is 1.317 Å, which is in excellent agreement with experimentally obtained bond length 1.314 Å. The optimized bond length C12–C13 involving the backbone for HF level is 1.4967 Å and is in agreement with the experimentally obtained value of 1.474 Å. The other calculated bond lengths and bond angles also show an excellent agreement with the X-ray data. Based on above comparison it can be said that although, there are some differences between the theoretical and experimental values, the optimized structural parameters can well reproduce the experimental ones and they are the basis for thereafter discussion.

The title molecule Deoxy-andrographolide has 54 atoms. The harmonic vibrational frequencies calculated for Deoxy-andrographolide at HF level using 3–21 basis set are listed in Table 5. Comparison of the frequencies calculated at HF/3–21 level with the experimental values (Table 5) reveals the overestimation of the calculated vibrational modes due to neglect of anharmonicity in real system. Vibrational frequencies calculated at HF/3–21 level were scaled by 0.89 [35].

Table 4
Optimised parameters for deoxy-andrographolide

S. no	Parameter	Bond length/Bond angle (angstrom/degree)	
		From HF-method	From X-ray data
1.	C1–C2	1.5359	1.518
2.	C1–C10	1.5487	1.587
3.	C2–C3	1.5253	1.494
4.	C3–C4	1.5490	1.545
5.	C3–O21	1.4354	1.459
6.	C4–C5	1.5646	1.572
7.	C4–C19	1.5363	1.542
8.	C4–C20	1.5475	1.516
9.	C5–C6	1.5437	1.487
10.	C5–C10	1.5736	1.538
11.	C6–C7	1.5429	1.553
12.	C7–C8	1.5123	1.505
13.	C8–C9	1.5246	1.510
14.	C8–C17	1.3191	1.326
15.	C9–C10	1.5796	1.590
16.	C9–C11	1.5453	1.519
17.	C10–C18	1.5438	1.503
18.	C11–C12	1.5409	1.489
19.	C12–C13	1.4967	1.474
20.	C13–C14	1.3177	1.314
21.	C13–C16	1.4945	1.483
22.	C14–C15	1.5165	1.498
23.	C15–O22	1.4501	1.450
24.	C16–O22	1.3737	1.339
25.	C16–O23	1.1948	1.212
26.	C19–O24	1.4567	1.434
27.	C2–C1–C10	113.1085	111.500
28.	C1–C2–C3	110.3985	110.600
29.	C2–C3–C4	112.2976	114.100
30.	C2–C3–O21	110.6908	108.800
31.	C4–C3–O21	113.0774	111.400
32.	C3–C4–C5	107.8451	108.100
33.	C3–C4–C19	112.0574	109.600
34.	C3–C4–C20	106.7427	110.500
35.	C5–C4–C19	112.5164	110.500
36.	C5–C4–C20	109.8447	108.900
37.	C19–C4–C20	107.6749	109.300
38.	C4–C5–C6	113.4807	113.400
39.	C4–C5–C10	117.383	119.200
40.	C6–C5–C10	111.3546	110.000
41.	C5–C6–C7	110.3601	111.300
42.	C6–C7–C8	109.9293	111.300
43.	C7–C8–C9	112.6020	114.500
44.	C7–C8–C17	121.5129	121.600

Table 4
(Continued)

S. no	Parameter	Bond length/Bond angle (angstrom/degree)	
		From HF-method	From X-ray data
45.	C9–C8–C17	125.8381	123.800
46.	C8–C9–C10	108.9368	108.500
47.	C8–C9–C11	113.5949	114.700
48.	C10–C9–C11	114.7701	113.800
49.	C1–C10–C5	108.3291	108.300
50.	C1–C10–C9	109.6595	107.700
51.	C1–C10–C18	109.1168	110.400
52.	C5–C10–C9	105.7242	106.600
53.	C5–C10–C18	115.2493	115.800
54.	C9–C10–C18	108.6396	107.600
55.	C9–C11–C12	111.5229	113.700
56.	C11–C12–C13	113.7513	115.500
57.	C12–C13–C14	132.2940	133.000
58.	C12–C13–C16	119.1339	119.500
59.	C14–C13–C16	108.5721	107.400
60.	C13–C14–C15	110.2308	110.400
61.	C14–C15–O22	103.3744	103.400
62.	C13–C16–O22	107.4971	109.100
63.	C13–C16–O23	128.8962	128.300
64.	O22–C16–O23	123.6067	122.500
65.	C4–C19–O24	107.9357	112.800
66.	C15–O22–C16	110.3255	109.600
67.	C10–C1–C2–C3	59.1536	61.900
68.	C2–C1–C10–C5	–51.2418	–51.900
69.	C1–C2–C3–C4	–60.8975	–61.400
70.	C2–C3–C4–C5	54.8689	50.600
71.	C3–C4–C5–C10	–50.5382	–45.200
72.	C10–C5–C6–C7	58.6379	60.500
73.	C4–C5–C10–C1	48.8470	46.300
74.	C5–C6–C7–C8	–54.8664	–51.600
75.	C6–C7–C8–C9	57.8542	55.3501
76.	C7–C8–C9–C10	–61.9312	–55.700
77.	C8–C9–C10–C5	60.6032	60.600
78.	C16–C13–C14–C15	0.012	–0.300
79.	C14–C13–C16–O22	–0.0436	–0.600
80.	C13–C14–C15–O22	0.0215	1.100
81.	C14–C15–O22–C16	–0.0494	–1.400
82.	C13–C16–O22–C15	0.0579	1.300

Carbonyl absorption. Carbonyl absorptions are sensitive and both the carbon and oxygen atoms of the carbonyl group move during the vibration and they have nearly equal amplitude. In the present study the C=O stretching vibration is observed at 1755 cm^{-1} and is in perfect agreement with the calculated frequency obtained at 1794 cm^{-1} .

C–H vibrations. The hetero aromatic structure shows the presence of C–H stretching vibrations in

Table 5
Comparison of the observed and calculated vibrational spectra of 14-deoxyandrographolide

HF	IR	Approximate mode description
3478	3905 (vw)	$\nu(\text{O-H})$ adj R1
3370	3277 (vs)	$\nu(\text{O-H})$ adj R1
3058	3072 (m)	$\nu(\text{C-H})$ R3
2967	2967 (s)	sym $\nu(\text{C-H})$ adj R2
2931	2932 (m)	asym $\nu(\text{C-H})$ adj R1 + asym $\nu(\text{C-H})$ R2
2869	2872 (m)	sym $\nu(\text{C-H})$ R1 + asym $\nu(\text{C-H})$ R2
2834	2364 (m)	$\nu(\text{C-H})$ R2
1794	1755 (vs)	$\nu(\text{C=O})$ adj R3
1665	1700 (w)	$\nu(\text{C=C})$ R3 + $\Phi(\text{H-C-C})$ adj R3
1662	1637 (m)	$\nu(\text{C=C})$ R2 + scis(H-C-H) adj R2
1523	1595 (m)	scis(H-C-H) adj R1
1446	1453 (s)	scis(H-C-H) adj R1
1386	1389 (w)	$\Phi(\text{C-C-H})$ cmn R1&R2 + $\Phi(\text{C-C-H})$ R1
1352	1351 (s)	$\Phi(\text{H-C-C})$ R1 + twist(H-C-H) BB
1297	1297 (m)	twist(H-C-H) BB + $\Phi(\text{H-C-O})$ adj R1
1274	1270 (sh)	twist(H-C-H) adj R1 + $\Phi(\text{H-C-C})$ R1
1213	1207 (s)	twist(H-C-H) R2 + $\Phi(\text{H-C=C})$ adj R2 + rock(H-C-H) adj R2
1149	1158 (m)	$\nu(\text{C-O})$ R3 + twist(H-C-H) R2 + $\Phi(\text{C=C-C})$ R3
1129	1130 (w)	rock(H-C-H) adj R1 + $\Phi(\text{H-O-C})$ adj R1
1070	1090 (vs)	$\Phi(\text{H-C-C})$ adj cmn R1&R2 + twist(H-C-C) R2 + $\Phi(\text{H-C-H})$ R1
1038	1038 (m)	rock(H-C-H) R3 + $\Phi(\text{C-C-O})$ R3 + $\Phi(\text{C-C-C})$ BB adj R3
946	952 (m)	twist(H-C-C) adj R2 + $\Phi(\text{C-C-C})$ adj R1 + $\Phi(\text{C-C-C})$ adj R1
899	902 (s)	def R2 + $\Phi(\text{C-C-C})$ BB adj R2 + $\Phi(\text{C-C-H})$ R3
849	844 (m)	rock(H-C-H) R1 + twist (H-C-H) adj R2 + puck R3
765	757 (w)	puck R3 + rock(H-C-H) adj R2 + $\tau(\text{C-C-C-C})$ BB
716	713 (w)	rock (H-C-H) adj R2 + $\omega(\text{H-C-C-C})$ R2 + $\tau(\text{H-C-C-H})$ R2
625	642 (w)	def R3 + $\tau(\text{C-C-C-C})$ BB adj R2 + $\omega(\text{C=C-C-})$ R2
570	565 (w)	rock R3 + $\Phi(\text{O=C-O})$ R3 + $\tau(\text{H-C-C-C})$ R2

Meaning of abbreviation: w – weak; vs – very strong; s – strong; m – middle; sh – shoulder; cmn – common to; adj – adjacent to; BB – back bone; ν – stretching; Φ – bending; τ – torsion; ω – wagging; def – deformation; rock – rocking; puck – puckering; twist – twisting; scis – scissoring.

the region 2800–3000 cm^{-1} which is the characteristic region for the ready identification of the C–H stretching vibration [36]. In the present study the C–H vibrations of the title compound are observed at 2967, 2872 cm^{-1} which is in perfect agreement with the calculated frequency at 2967 and 2869 cm^{-1} , respectively.

O–H vibrations. The precise position of O–H band is dependent on the strength of hydrogen bond. The O–H stretching is observed at 3277 cm^{-1} and is calculated at 3370 cm^{-1} in the hydrogen bonded systems. Almost in all vibrational modes overestimation is noticed at HF level due to the single wave function and the neglect of electron correlation.

5. Conclusion

Ab initio calculations using Hartree–Fock method and 3–21 basis set was performed on the title molecule for the first time with the Gaussian 03 software. The equilibrium geometries and harmonic frequen-

cies of deoxy-andrographolide were determined and analysed at both MM and QM (HF) level of theories. The vibrational frequency calculation proved that the structure is stable (no imaginary frequencies). The difference between the observed and the calculated value of frequency of most of the fundamentals is very small. The Potential energy distribution contribution to each of the observed frequency (in MM calculations) shows the reliability and accuracy of the normal mode analysis. The normal mode analysis of deoxy-andrographolide shows a dynamical behaviour and possibly, opens up an avenue for further conformational research. With the continuing need for novel structures and the difficulty of gaining access to large tracts of bio diversity in habitats, combinatorial chemistry blended with modern quantum chemical methods can prove to be blessing for the researchers.

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