J Chem Crystallogr (2011) 41:697–702
DOI 10.1007/s10870-010-9956-7

ORIGINAL PAPER

Synthesis and Structural Proof of a Potent 5-Lipoxygenase Inhibitor

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Received: 28 April 2010 / Accepted: 30 December 2010 / Published online: 19 January 2011

Abstract 5-Lipoxygenase inhibitor 3-*O*-acetyl-9,11-dehydro-β-boswellic acid was detected in the extract of Boswellia serrata gum resulting from unstable 11-hydroxy precursor. It was reported more potent than other Boswellic acids in its inhibition of 5-Lipoxygenase. Here, we report the method of conversion of 3-acetoxy-β-boswellic acid to 3-*O*-acetyl-9,11-dehydro-β-boswellic acid, and the crystal structure of later. This compound crystallizes in orthorhombic space group $P2_12_12_1$ with cell parameters of a = 12.726(1) Å, b = 16.597(1) Å, c = 27.332(2) Å, $\alpha = \beta = \gamma = 90°$, V = 5772.7(5) Å^3, D_c = 1.143 Mg/m^3, and $Z = 8$. The X-ray structure investigation indicates that the rings A, B, D and E are exhibit chair and the ring C adopts a distorted half chair conformation. The conformational difference of the two structures in the arrangement is due to crystal packing of 3-*O*-acetyl-9,11-dehydro-β-boswellic acid. The molecular packing is stabilized by C–H···O and O–H···O types of hydrogen bonding interactions.

Keywords Boswellia serrata gum · 5-Lipoxygenase inhibitor · Medicinal activity · Hydrogen bonding

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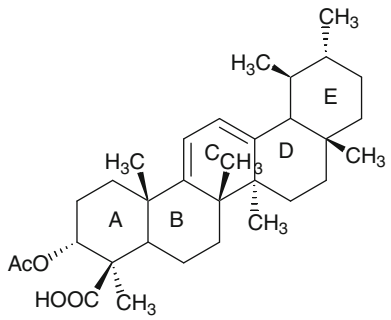
Introduction

Boswellia serrata gum has been a well recognized natural product for its anti-inflammatory action [1]. The most active material in the gum was identified [2], to be 3-acetoxy-11-keto-β-boswellic acid (**1** AKBBA). The mode of action of AKBBA has been traced towards its novel non-redox, allosteric inhibition of 5-Lipoxygenase preventing the formation of inflammatory mediators leukotrienes [3]. The crude gum, in addition to AKBBA and related acids, contains several other terpenoid components often possessing either inhibitory or potentiating effects on 5-Lipoxygenase. Hence increasing attention is paid towards characterizing other constituents of Boswellia serrata gum.

Safayhi et al. identified one such artifact in their study [4], wherein 3-*O*-acetyl-9,11-dehydro-β-boswellic acid (**2**) (Scheme 1) as also a 5-Lipoxygenase inhibitor, actually more potent than AKBBA. The formation of (**2**) in the extract was traced towards an unstable 11-hydroxy precursor molecule in the gum leading to the formation of (**2**). Since increasing attention is paid towards the standardization of activity of crude plant drugs, it is important to secure the structure of (**2**). In this communication we describe the conversion of 3-acetoxy-β-boswellic acid (**3**), an independently well characterized molecule by single crystal X-ray analysis [5], to 3-*O*-acetyl-9,11-dehydro-β-boswellic acid (**2**) chemically thus firmly securing the structure of (**2**). In addition a single crystal X-ray structure of (**2**) independently establishes structure of (**2**) now available through this study for biological studies.

Thus 3-acetoxy-β-boswellic acid (**3**) on reaction with N-bromosuccinimide [6], directly gave (**2**) in good yields. Hydrolysis of the acetate (**2**) furnished (**4**). We also found



Scheme 1 Chemical structure of 3-*O*-acetyl-9,11-dehydro-β-boswellic acid

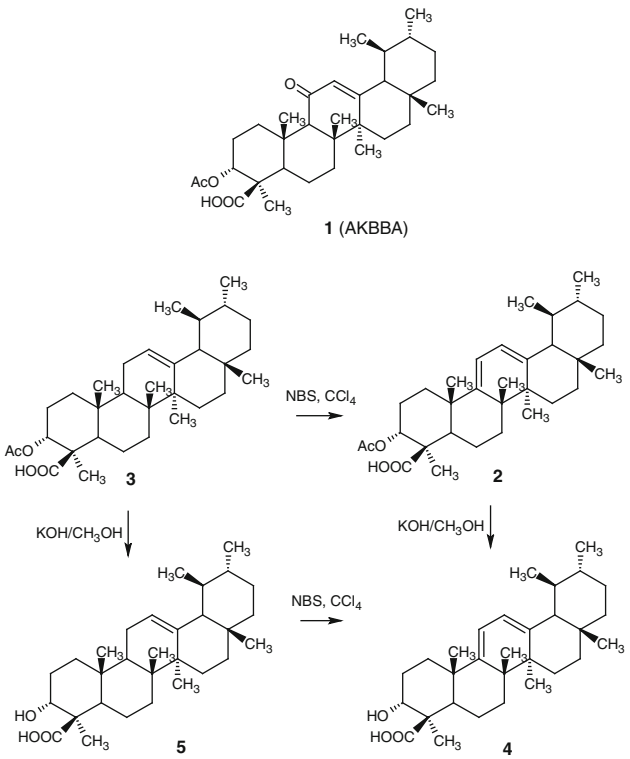


Fig. 1 Scheme of compounds **1**, **2**, **3**, **4** & **5**

that β-boswellic acid (**5**) itself could be converted to 3-hydroxy-9,11-dehydro-β-boswellic acid (**4**), without any protection of the 3-OH function (Fig. 1).

Experimental

Melting points reported are uncorrected. Elemental analysis was done at Robertson Microlit Laboratories, Madison, NJ. Proton NMR spectrum were taken at 500 MHz in a CDCl3 solvent with TMS as internal standard.

Synthesis of 3-*O*-Acetyl-9,11-dehydro-β-boswellic Acid (**2**)

3-Acetyl-β-boswellic acid (2.0 g) was taken in carbontetrachloride (40 mL). N-Bromosuccinimide (1.2 g) was added and the reaction mixture was refluxed for 4–5 h. TLC indicated completion of the reaction. The reaction mixture was washed twice with water. The organic layer was dried over sodium sulfate and the solvent was removed to yield a crude product weighing 1.6 g; this crude product was dissolved in methanol (60 mL) and ethyl acetate (10 mL) and charcolized. The solvents were removed to yield a light brown powder weighing 1.2 g which was further purified by column chromatography to yield a white material (0.7 g). Mp: 290–292 °C (reported [4] 230 °C). Elem. Analysis (calc. values for $C_{32}H_{48}O_4$ in parenthesis) %C: 77.48 (77.38), %H: 9.86 (9.74). ^{1}H NMR ($CDCl_3$ solvent): 5.66 (1H, d, $J = 5.87$ Hz), 5.47 (1H, d, $J = 5.87$ Hz), 5.32 (1H, distorted t, $J = 3.04$ Hz), 2.18 (1H, br t, $J = 13.8$ Hz), 2.10–2.00 (4H, including a singlet at 2.07 for 3H, CH_3–CO and a, d of t centered at 2.03, $J = 13.3$ & 4.4 Hz for 1H), 1.98–1.86 (2H, d of q, $J = \sim 12.5$ & ~ 4.5 Hz), 1.86–1.67 (4H, m), 1.62–1.25 (13H, m, including a singlet for 3H at 1.28 for a CH_3), 1.20 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.07 (1H, br d, $J = 11.6$ Hz), 1.00–0.91 (m, 8H, including a singlet at 0.95 for 3H for a –CH_3 and another broad singlet at 0.94 for 3H for a CH–CH_3), 0.87 (3H, s, CH_3), 0.82 (3H, d, $J = 6.2$ Hz, CH–CH_3)

3-Hydroxy-9,11-dehydro-β-boswellic Acid (**4**)

3-*O*-Acetyl-9,11-dehydro-β-boswellic acid (**2**) (3.5 g) was refluxed in methanol (70 mL) in the presence of aq. KOH (5 g in 5 mL water) till the completion of reaction as indicated by TLC. The reaction time was about 6 h. Water (70 mL) was added and the pH was adjusted to 2–3 range using dil. HCl. The reaction mixture was stirred well and filtered. The solid was washed with water till neutral pH and dried at 80 °C to yield 3.2 g of the product. One gram of this product was further purified by column chromatography to obtain 0.8 of analytically pure product. Mp: 240–245 °C (reported [4] 218 °C). Analysis (calc. values for $C_{30}H_{46}O_3$ in parenthesis) %C: 79.28 (79.25), %H: 10.40 (10.20). ^{1}H NMR ($CDCl_3$ solvent): 5.66 (1H, d, $J = 5.87$ Hz), 5.46 (1H, d, $J = 5.87$ Hz), 4.09 (1H, distorted br t), 2.25 (1H, br t, $J = 14.5$ Hz), 2.02(1H, d of t, $J = 13.2$ & 4.4 Hz), 1.97–1.86 (2H, m), 1.84–1.55 (7H, m), 1.54–1.45 (2H, m), 1.43–1.35 (5H, m including a singlet at 1.38 for 3H for a –CH_3), 1.24–1.35 (4H, m), 1.19 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.06 (1H, broad d, $J = 11.7$ Hz), 0.98–0.90 (8H, m, including a broad singlet

at 0.93 for 6H for two $-CH_3$), 0.86 (3H, s, CH_3), 0.80 ((3H, d, $J = 6.3$ Hz, CH–CH_3)

3 → **5** and **2** → **4** conversions utilized similar procedures described above.

Single Crystal X-Ray Diffraction

The 3-*O*-Acetyl-9,11-dehydro-β-boswellic acid (**2**) compound has been crystallized from ethanol by slow evaporation technique at room temperature yielded a white rectangular block shaped single crystals. X-ray intensity data collection was performed at room temperature. All the diffraction intensity measurements were made on Bruker's APEX-II CCD Area Detector [7], with graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). The data has been corrected for Lorentz and Polarization effects but not for absorption and extinction. The structure was solved by direct methods and refined by full-matrix least-squares using SHELXS97 [8], and SHELXL97 [9] programs. During the refinement, all non-hydrogen atoms were treated anisotropically and the H-atoms were fixed geometrically and refined as a riding model gave final residual index $R(F) = 0.037$ and $wR(F^2) = 0.101$ for 5628 observed reflections [$I > 2\sigma(I)$]. The crystal data and the refinement results are given in Table 1. Selected bond lengths, bond angles and torsion angles of (**2**) are listed in Table 2. The fractional atomic coordinates of both molecules of the asymmetric unit and their detailed geometry have been deposited to Cambridge Crystallographic Data Centre (CCDC-657235) as a supplementary publication.

Table 1 Crystal data and the structure refinement of 3-*O*-acetyl-9,11-dehydro-β-boswellic acid

Empirical formula	$C_{32}H_{48}O_4$
CCDC No.	657235
Formula weight	496.7
Temperature	273(2) K
Radiation type and wavelength	MoKα; 0.71073 Å
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Unit cell dimensions	$a = 12.726(1)$ Å
	$b = 16.597(1)$ Å
	$c = 27.332(2)$ Å
Volume	5772.7(5) Å^3
Z, Calculated density	8, 1.143 Mg/m^3
Absorption coefficient	0.073 mm^{-1}
$F(000)$	2176
Crystal shape and colour	Rectangular block, colourless
Crystal size	0.17 × 0.1 × 0.07 mm
Theta range for data collection	1.77–25.59°
Limiting indices	$h = -15 \rightarrow 15$, $k = -19 \rightarrow 19$, $l = -32 \rightarrow 32$
Reflections collected/unique	41973/5628
Refinement method	Full-matrix least-squares on F^2
Goodness-of-fit on F^2	1.033
Final R indices [$I > 2\sigma(I)$]	$R(F) = 0.037$, $wR(F^2) = 0.101$
Largest diff. peak and hole	0.18 and −0.13 eÅ^{-3}

Table 2 Comparison of geometrical parameters (Å,°) of C-ring of structure (**2**) with similar ring of the analogous structure (**3**)

	Molecule-I	Molecule-II
Bond lengths		
C(9)–C(10)	1.535(3)	1.537(3)
	1.542	1.556
C(9)–C(11)	1.581(3)	1.582(3)
	1.598	1.601
C(10)–C(14)	1.334(3)	1.334(3)
	1.528	1.530
C(11)–C(12)	1.533(3)	1.539(3)
	1.533	1.529
C(12)–C(13)	1.330(3)	1.325(3)
	1.331	1.325
C(13)–C(14)	1.451(4)	1.452(3)
	1.488	1.502
Bond angles		
C(10)–C(9)–C(11)	109.0(2)	109.5(2)
	108.4	106.3
C(14)–C(10)–C(9)	115.4(2)	116.5(2)
	110.5	109.6
C(12)–C(11)–C(9)	109.3(2)	110.0(2)
	109.5	109.2
C(13)–C(12)–C(11)	116.8(2)	116.5(2)
	119.8	119.6
C(12)–C(13)–C(14)	122.7(2)	123.1(2)
	126.8	126.4
C(10)–C(14)–C(13)	122.8(2)	122.8(2)
	114.1	113.9
Torsion angles		
C(10)–C(9)–C(11)–C(12)	54.8(2)	52.4(2)
	55.7	59.4
C(11)–C(9)–C(10)–C(14)	−42.2(3)	−37.6(3)
	−61.3	−64.2
C(9)–C(10)–C(14)–C(13)	7.4(4)	4.1(4)
	36.3	36.6
C(10)–C(14)–C(13)–C(12)	16.9(5)	16.4(3)
	−5.6	−3.06
C(14)–C(13)–C(12)–C(11)	−0.6(4)	2.2(3)
	−0.8	1.2
C(13)–C(12)–C(11)–C(9)	−34.7(3)	−36.1(3)
	−26.4	−28.1

Table 3 Hydrogen bonding (Å,°)

D–H···A	D–H	H···A	D···A	D–H···A
C(21′)–H(21′)…O(1)^{i}	0.98	2.97(2)	3.831(3)	147.0(2)
O(2′)–H(2′)···O(1)^{ii}	0.82	1.90(2)	2.711(2)	168.6(1)
O(2)–H(2)···O(3′)^{iii}	0.82	1.90(2)	2.689(3)	161.0(2)
C(4)–H(4A)···O(1′)^{iv}	0.97	2.77(2)	3.602(4)	144.4(2)
C(27)–H(27B)···O(1)	0.96	2.75(2)	3.526(3)	138.5(2)
C(27′)–H(27F)···O(1′)	0.96	2.81(2)	3.605(3)	140.8(2)

Symmetry codes: (i) $x + 1/2, -y + 3/2, -z$ (ii) $x, y - 1, z$ (iii) $-x + 1, y + 1/2, -z + 1/2$ (iv) $x, y + 1, z$

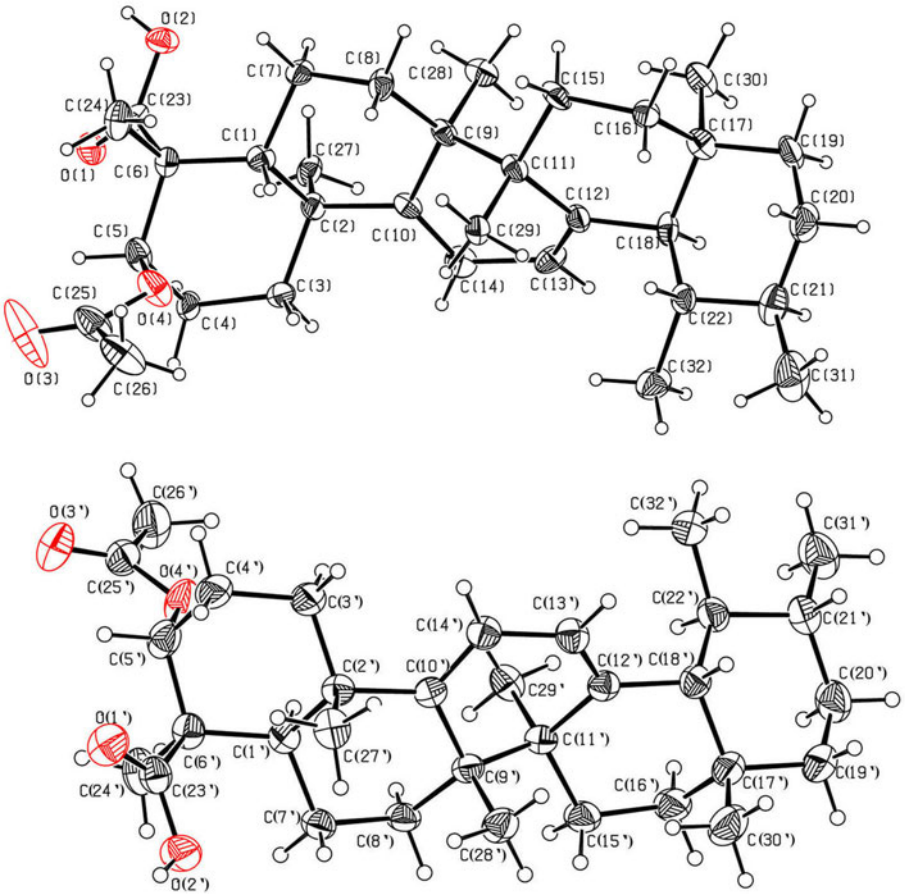


Fig. 2 View of the molecule (**2**) showing atom-labeling scheme with thermal ellipsoid atoms drawn at 50% probability level, H atoms are drawn as small circles of arbitrary radii

Results and Discussion

The title compound 3-*O*-Acetyl-9,11-dehydro-β-boswellic acid (**2**) crystallizes two molecules in the asymmetric unit as its analogous [5]. Figure 2 is the ORTEP [10] plot of molecular structure, showing, thermal ellipsoid atoms with atom numbering scheme. Although, the compound crystallizes two molecules in the asymmetric unit, their bond lengths and angles are approximately same. The different types of bond distances in the molecules I and II and their ranges are; C_{sp3}–C_{sp3}: 1.504(3) to 1.581(3) [1.502(4) to 1.582(3)*] Å (* indicates [bond lengths/angles of molecule (**2**)]), C_{sp2}–C_{sp3}: 1.523(3) to 1.545(3) [1.525(3) to 1.550(3)*] Å and C_{sp2}–C_{sp2}: 1.330(3) to 1.451(4) [1.325(3) to 1.452(4)*] Å. The observed major difference in bond angles are around the atoms C(10), C(13), C(14) and C(15); this difference is attributed to the conformation of C-ring. The C(16)–C(17)–C(18) angle is 107.6(2)° [108.3(2)°]* which is small on compared with all other bond angles of the rings in the molecules. This may be due to the presence of bulk groups attached at C(6) and C(17). The carbonyl bonds C(23)–O(2) and C(25)–O(2) are

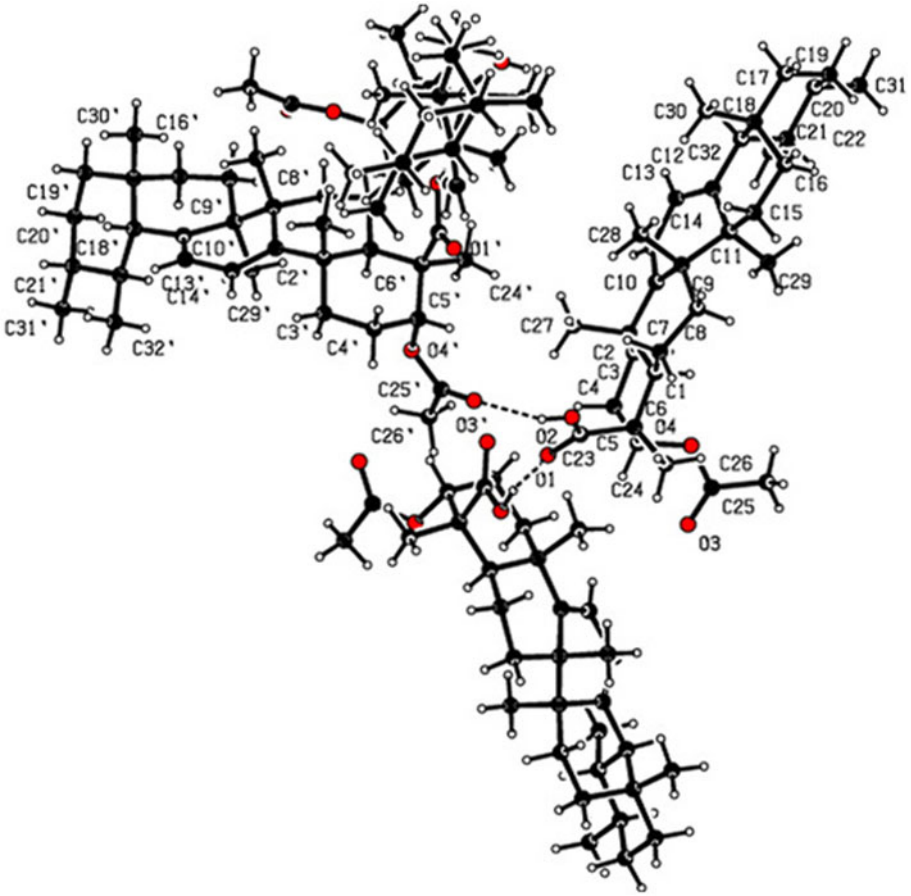


Fig. 3 Hydrogen bonding interactions

significantly shorter than the similar type of bonds C(5)–O(4): 1.451(3) [1.461(3)*] Å, C(23)–O(2):1.324(3) [1.324(3)*] Å and C(25)–O(4): 1.319(4) [1.323(4)*] Å. As expected, the double bond distances C(25)–O(3): 1.179(5) [1.192(5)*] Å and C(23)–O(1): 1.203(3) [1.198(3)*] Å in the molecule are almost equal. However, both the molecules exhibit almost similar geometry, but they are conformationally slightly different. This is due to the effect of the mode of crystallization. Similar trend appears in the reported structure (**3**) [5]. The geometry and conformation of both (**2**) and (**3**) are differing by C-ring. These conformational differences can be determined from their torsion angles. We have analyzed individual rings of (**2**) and (**3**), on the basis of formalism reported by DUAX et al. [11]. The average torsion angles of ring A of I and II in the asymmetric unit are 54.2 and 53.6°, respectively, indicates, a chair conformation and further the rings B, D and E also exhibit the same; but unlikely the ring C adopt distorted half chair conformation [12]. Table 2, shows the difference in torsion angles of C-ring of (**2**) and (**3**). Precisely, the C-ring altered the molecular conformation significantly; perhaps, the enhanced medicinal activity of the structure (**2**) may be attributed to the C-ring geometry.

Figure 3 depicts the hydrogen bonding interactions of the molecules in the crystal. The molecular packing is predominantly stabilized by C–H···O and O–H···O type of hydrogen bonding interactions. Among the C–H···O type of interactions, the C(21′)–H(21′)···O(1)^{i} interaction is considered as a strong interaction and the H-bond parameters are; C(21)–H(21′): 0.980 Å, H(21′)···O(1)^{i}: 2.970(2) Å, C(21)···O(1)^{i}: 3.831(3) Å and the angle is C(21′)–H(21′)···O(1)^{i}: 147.0(2)°. The O(2′)–H(2′)···O(1)^{ii} hydrogen bonding parameters are; O(2′)–H(2′): 0.820 Å, H(2′)···O(1)^{ii}: 1.900(2) Å, O(2′)···O(1)^{ii}: 2.711(2) Å and the angle is O(2′)–H(2′)···O(1)^{ii} : 168.6(1)°. [Symmetry codes: (i) $x + 1/2, -y + 3/2, -z$ (ii) $x, y - 1, z$]. Some of the weak inter and intra molecular interactions are listed in Table 3.

Supporting Information

CCDC-657235 contains the supplementary crystallographic data of this paper. These data can be obtained from the Cambridge crystallographic data centre (CCDC), 12, Union Road, Cambridge CB2 1EZ, UK.

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