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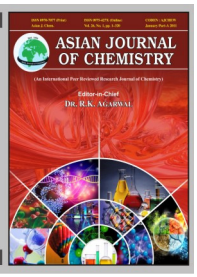

Isolation and Spectral Assignments of Lipoxygenase Inhibiting Triterpene from *Solanum surrattense*

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A lipoxygenase inhibiting triterpene have been isolated for the first time from the ethyl acetate soluble fraction of whole plant of *Solanum surrattense* and the structure was assigned on the basis of 1D (^{1}H NMR, ^{13}C NMR, DEPT, NOE) and 2D NMR (HMBC, HMQC, COSY, NOESY) experiments. The biological potential of the isolated compound was performed against lipoxygenase enzyme using baicalein as a positive control.

Keywords: Triterpene, *Solanum surrattense*, Lipoxygenase enzyme.

INTRODUCTION

The genus *solanum* comprises about 3000 species, is widely distributed in the tropical region of Asia, Australia and Polynesian Islands. In Pakistan, it is common in waste places from planes to 1500 M. *Solanum surrattense* is a perennial prickly prostrate herb and have been used in folk medicine for the treatment of bronchial asthma, non specific cough, vomiting, catarrhal fever, rheumatism, diarrhea, blood cancer and controlling of stones in bladders. The plant is bitter, digestive, alterative, astringent, expectorant, aperients and carminative[1]. The pharmacological importance of the *Solanum surrattense* prompted us to investigate the phytochemicals from the ethyl acetate soluble fraction of the whole plant. Our detailed investigations have led to the discovery of a compound **1**, assigned as 3β-benzoyloxy-12(13)-ursene-11-one and 1β,3β,22α*N*-spirosol-5-ene, respectively. The compound was identified by IR, MS, 1D and 2D NMR spectroscopic techniques.

EXPERIMENTAL

Melting points were obtained on Buchi melting point apparatus and uncorrected. Optical rotations were taken on a JASCO DIP 360 polarimeter. The IR spectra were recorded on a FTIR-8900 Shimadzu spectrometer. The 1D and 2D NMR spectra were recorded in $CDCl_3$ at 500 MHz on a Bruker Av 500 spectrometer. The chemical shift values are reported in ppm (δ) units and coupling constant (*J*) are shown in Hz. EIMS, HREIMS and HRFABMS were recorded on a JMS-HX-110 with a data system on JMS-DA 500 mass spectrometer. Aluminum sheets pre-coated with silica gel $60F_{254}$ (20 cm × 20 cm, 0.2 mm thick, E-Merck) were used for TLC and silica gel (230-400 mesh) was used for column chromatography. Pre-coated RP-18 gel (E-Merck) glass plates were used for TLC. GC-18A equipped with FID was used for GLC. For lipoxygenase (1.13.11.12) type 1-B (Soybean) inhibitory assay all chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

The dried (whole plant, 10 kg) *S. surrattense* Burm. f. was collected from cholistan desert near bahawalpur in June 2006 and identified by Dr. Muhammad Arshad, Plant Taxonomist, Cholistan Institute of Desert Studies, (CHIDS), The Islamia University of Bahawalpur, where a voucher specimen (CHIDS / 01 / 05) has been deposited.

Extraction and isolation: The shade dried 10 kg crushed plant was chopped and soaked in methanol (3 × 25 mL) for thrice and the combined methanolic extracts were concentrated under reduced pressure to give a crude extract (800 g). The crude extract was portioned between *n*-hexane (125 g), $CHCl_3$ (80 g), EtOAc (65 g) and *n*-ButOH (155 g) extract. The ethyl acetate portion was applied to silica gel column chromatography

and eluted with $CHCl_3$-methanol in increasing order of polarity to obtain 12 fractions. The fraction 3 (1.58 g) showed a major spot TLC plate under UV 254 nm light and was again chromatographed over silica gel using the same solvent system ($CHCl_3$-methanol) in increasing order of polarity to obtain 6 fractions. The fraction 4, obtained from $CHCl_3$-methanol (60:40) was chromatographed over preparative TLC using solvent system ethyl acetate-methanol (70:50, total volume 10 mL) to obtain **1** (R_f = 0.6) in a 22 mg amount.

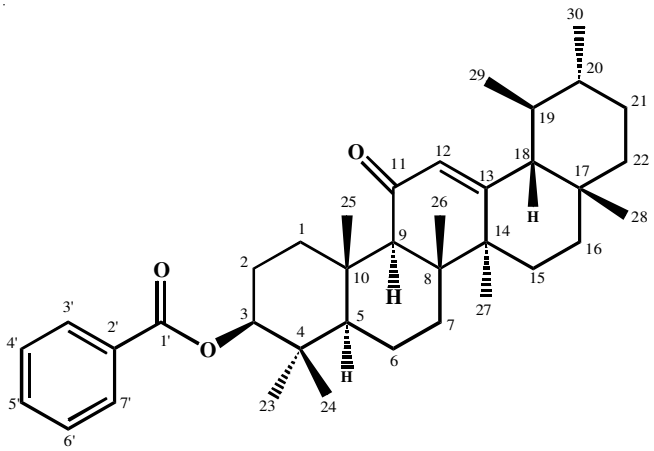
Spectroscopic data: 3β-Benzoyloxy-12(13)-ursene-11-one (**1**), amorphous solid, m.p. 276-276.5 °C, $[\alpha]_D^{20}$ + 106° (c = 1 MeOH). IR ν_{max}, cm^{-1}, 1731, 1650, 1605. EIMS *m/z*: [M^+] 544 (16), 440 (70), 312 (100 %), 297 (28), 257 (94), 243 (20), 105 (6). HREIMS M^+ *m/z* 544.3910, (calcd. for $C_{37}H_{52}O_3$, 544.3903). ^1H and ^{13}C-NMR, Table-1.

***in vitro* Lipoxygenase inhibition assay:** LOX inhibiting activity was measured by modifying the spectrophotometric method developed by Tappel[2] and already described in our previous work[3]. Reaction mixtures containing 160 µL (100 mM) sodium phosphate buffer (pH 8), 10 µL of test-compound solution and 20 µL of lipoxygenase solution were mixed and incubated for 10 min at 25 ºC. The reaction was then initiated by the addition of 10 µL linoleic acid (substrate) solution, with the formation of (9Z,11E)-(13S)-13-hydroperoxy-octadeca-9,11-dienoate and the change of absorbance at λ_{max} 234 nm was followed for 6 min. All the inhibition experiments were performed in triplicate in 96-well micro-plates in spectra max 340 (Molecular Devices, U.S.A). The IC_{50} values were then calculated using the EZ-Fit Enzyme kinetics program (Perrella Scientific Inc. Amherst, U.S.A). The percentage (%) inhibition was calculated as follows (E-S)/Ex 100, where E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound.

RESULTS AND DISCUSSION

The methanolic extract of *S. surrattense* was partitioned successively with *n*-hexane, chloroform, ethyl-acetate, *n*-butanol and water soluble fractions. The ethyl acetate layer was dried and subjected to various chromatographic techniques to afford **1**.

Compound (**1**) was isolated as amorphous solid in a 22 mg amount. The EIMS spectrum gave the molecular ion peak at *m/z* 544 while the HREIMS assigned the molecular formula $C_{37}H_{52}O_3$ (M^+; *m/z* 544.3910, calcd. for $C_{37}H_{52}O_3$: 544.3903) The IR spectrum showed an a, β-unsaturated six membered ring ketone, aromatic and ester moiety at ν_{max} 1650, 1605 and 1731 cm^{-1} respectively in the molecule. The ^1H NMR displayed six tertiary methyls as singlets at δ 0.90, 1.00, 1.15, 1.34, 1.25 and 0.82 and two secondary methyls as doublet at δ 0.94 (*J* = 7.2 Hz) and 0.81 (*J* = 7.5 Hz). A characteristic oxymethine proton appeared at δ 4.79 (dd, *J* = 9.1, 7.7 Hz) and olefinic proton at δ 5.41 as singlet indicated the ursane type of skeleton. Further more the ^1H NMR displayed aromatic signals at δ 7.94 (dt, *J* = 7.7, 2.1 Hz), δ 7.45 (dt, *J* = 7.2, 2.3 Hz) and δ 7.54 as a multiplet showed the attachment of aromatic moiety with the molecule. The ^{13}C NMR spectrum displayed thirty seven signals in BB spectra while DEPT spectrum sorted out as eight methyls (δ 21.4, 23.4, 16.3, 18.5, 22.9, 28.7, 17.4 and 21.3),



Structure of compound **1**

eight methylenes (δ 39.2, 23.8, 17.4, 32.6, 26.7, 28.1, 31.2 and 41.5), twelve methines (δ 79.4, 54.2, 61.1, 125.4, 58.4, 38.8, 39.6, 129.7, 128.7 and 132.9) and nine quaternary carbons (δ 130.9, 166.5, 39.0, 43.5, 37.0, 200.0, 164.0, 42.7 and 35.1).

The downfield signals at δ 200 and 166.5 were due to carbonyl carbons while olefinic carbons were appeared at δ 164, 130.9 and 128.7. The signal at δ 79.4 could be assigned to oxymethine carbon. This indicated that (**1**) is a Δ^{12} ursene type of triterpene in addition to the presence of an oxo and benzoyl moiety. In ^1H-^1H-COSY spectrum the carbinylic proton at δ 4.79 showed correlation with two other protons limiting its presence either to C-1 or C-3. By HMBC it could be assigned to C-3, as it showed ^2J correlation with C-2 (δ 23.8) and C-4 (δ 39) and ^3J correlation with C-23 (δ 21.4) C-24 (δ 23.4), C-5 (δ 54.2), C-1 (δ 39.2) and carbonyl of benzoyl moiety C-1' (δ 166.5). The larger coupling constant value assigned this proton in an α and axial configuration. The remaining problem was to assign the position of carbonyl group. This was confirmed by the presence of ^1H NMR signal assigned to H-9 at δ 2 (1H, s, H-9). In ^{13}C NMR spectrum the strong deshielding signals for C-9 (δ 61.1), C-11 (δ 125.4) and C-13 (δ 164) indicated its presence at δ C-11[4-7]. Other important correlations in HMBC were observed for H-12 at δ 5.34 with C-11 (δ 200), C-13 (δ 164) and C-9 (δ 61.1), C-14 (δ 42.7), C-18 (δ 58.4) as ^2J and ^3J respectively, further confirming the presence of oxo group at C-11. The stereochemistry was confirmed by NOESY effects in which H-3 showed correlation with H_3-23 and H-5, H-18 with H-19, H-28 indicating H-18 in a β configuration. This was confirmed by the presence in the ^1H NMR spectrum of signals assigned to H-9 at δ 2 (1H, s, H-9). Thus compound **1** was determined as 3β-benzoyloxy-12(13)-ursene-11-one (Table-1). This is the first report of the natural occurrence of this triterpene, following its earlier synthesis[8].

Lipoxygenases (LOXs, EC 1.13.11.12) comprise a category of non-heme iron containing dioxygenases. In animals and plants dioxygenases are widely distributed. For the biosynthesis of several bioregulatory compounds, hydroxyeicosatetraenoic acids, leukotrienes, hepoxylines and lipoxins in mammalian cells the lipoxygenases play important role[9]. It was considered as diversity of disorders in mammalian cells such as bronchial asthma, inflammation and the development

TABLE 1
1D (^{1}H, ^{13}C NMR) AND 2D NMR (HMQC, HMBC, NOESY) DATA OF COMPOUND **1** IN $CDCl_3$

Position no	^{1}H[a] (HMQC)	Multiplicity and *J* (Hz)	^{13}C[b]	HMBC[c] (H → C)
1	1.90	m	39.2	J^2 (C-2, C-10)
	2.00			J^3 (C-3, C-5, C-25)
2	1.90	m	23.8	J^2 (C-1, C-3)
	1.80			J^3 (C-4, C-10, C-23, C-24)
3	4.81	dd, (9.8, 7.1)	79.4	J^2 (C-2, C-4)
				J^3 (C-1, C-5, C-1′)
4	-	-	39.0	-
5	1.02	m	54.2	J^2 (C-4, C-6, C-10)
				J^3 (C-7, C-10, C-23, C-10, C-25)
6	1.70	m	17.4	J^2 (C-5, C-7)
	1.85			J^3 (C-4, C-8, C-10
7	1.70	m	32.6	J^2 (C-6, C-8)
	1.48			J^3 (C-5, C-9, C-14, C-25)
8	-	-	43.5	-
9	2.35	s	61.1	J^2 (C-8, C-10, C-11)
				J^3 (C-1, C-7, C-5, C-12, C-14, C-25)
10	-	-	37.0	-
11	-	-	200.0	-
12	5.41	s	125.4	J^2 (C-11, C-13)
				J^3 (C-9, C-14, C-18)
13	-	-	164.0	-
14	-	-	42.7	-
15	1.87	m	26.7	J^2 (C-14, C-16)
	1.99			J^3 (C-13 C-17, C-27)
16	2.04	m	28.1	J^2 (C-15, C-17)
	2.17			J^3 (C-14, C-18, C-28)
17	-	-	35.1	-
18	1.34	d, (7.7)	58.4	J^2 (C-13, C-17, C-19)
				J^3 (C-12, C-14, C-28, C-29)
19	1.52	m	38.8	J^2 (C-18, C-20, C-29)
	1.57			J^3 (C-13, C-17, C-21, C-30)
20	1.00	m	39.6	J^2 (C-19, C-21, C-30)
	1.10			J^3 (C-18, C-22, C-29)
21	1.29	m	31.2	J^2 (C-20, C-22)
	1.80			J^3 (C-17, C-19, C-30)
22	1.14	m	41.5	J^2 (C-17, C-21)
	1.11			J^3 (C-18, C-16, C-20, C-28,)
23	0.93	S	21.4	J^2 (C-4)
				J^3 (C-3, C-5, C-24)
24	0.93	S	23.4	J^2 (C-4)
				J^3 (C-3, C-5, C-23)
25	1.14	S	16.3	J^2 (C-10)
				J^3 (C-1, C-5, C-9)
26	1.34	S	18.5	J^2 (C-8)
				J^3 (C-9, C-14)
27	1.29	S	22.9	J^2 (C-14)
				J^3 (C-8, C-13)
28	0.81	S	28.7	J^2 (C-17)
				J^3 (C-16, C-22)
29	0.94	d, (7.9)	17.4	J^2 (C-19)
				J^3 (C-18, C-20)
30	0.81	d, (6.9)	21.3	J^2 (C-20)
				J^3 (C-19, C-21)
1′	-	-	166.5	-
2′	-	-	130.9	-
3′	7.94	ddd (7.4, 2.2, 1.5)	129.9	J^2 (C-2′, C-4′)
				J^3 (C-1′, C-5′, C-7′)
4′	7.45	M	128.7	J^2 (C-3′, C-5′)
				J^3 (C-2′, C-6′)
5′	7.54	M	132.9	J^2 (C-4′, C-6′)
				J^3 (C-3′, C-7′)
6′	7.45	M	128.7	J^2 (C-5′, C-7′)
				J^3 (C-2′, C-4′)
7′	7.94	ddd,(7.4, 2.2, 1.5)	129.9	J^2 (C-2′, C-6′)
				J^3 (C-1′, C-3′, C-5′)

[a] ^{1}H NMR carried out at 400 MHz, [b] ^{13}C NMR carried out at 100 MHz, [C] HMBC carried out at 400 MHz, [d] HMBC carried out at 400 MHz

of several human cancers were due to the production of LOXs. Furthermore it was considered as LOXs are the potential targets for drug design and the discovery of new inhibitors for the treatment of inflammation and bronchial asthma diseases[3,9]. The isolated compound **1** showed moderate inhibitory activity against lipoxygenase (Table-2).

TABLE-2
in vitro QUANTITATIVE INHIBITION OF LIPOXYGENASE BY COMPOUND **1**

Compound	IC_{50} ± SME[a] [μm]
1	58.5 ± 0.10
Baicalein[b]	8.0 ± 0.04

[a]Standard mean error (SEH) of three experimental determinations.
[b]Positive control used in assays.

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