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BRAHIN, A NEW LIPOXYGENASE INHIBITING TRITERPENE FROM *Spiraea brahuica*

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*Brahin (**1**), a new ursene-type triterpene, has been isolated from the $CHCl_3$-soluble fraction of* Spiraea brahuica *together with* β*-sitosterol (**2**), 3-(3,4-dimethoxyphenyl)-2-propenal (**3**), and cinnamic acid (**4**), which are reported for the first time from this species. The structures of these compounds were elucidated by 1D and 2D NMR spectroscopic techniques. Brahin showed moderate inhibitory activity against the enzyme lipoxygenase.*

Keywords: *Spiraea brahuica*, Rosaceae, ursene-type triterpene, brahin.

Spiraea is one of the most important genus of the family Rosaceae. It comprises 80–100 species that grow as shrubs in the northern hemisphere, with the greatest diversity in eastern Asia [1]. These contain hetisine-and atisine type diterpene alkaloids, flavonoids, cinnamoyl and benzaldehyde derivatives, most of which show pharmacological properties [2, 3]. One of the species of genus *Spiraea* is *S. brahuica* Boiss., which is distributed in Asia. In Pakistan, it abundantly grows in Ziarat Valley of the Province of Balochistan [4]. Previously, flavonoids have been reported by us from this species [5]. The ethnopharmacological and chemotaxonomic importance of the genus *Spiraea* prompted us to further investigate the chemical constituents of *Spiraea brahuica*. Herein we report the isolation and structural elucidation of a new ursene-type triterpene, named brahin (**1**), along with *β*-sitosterol (**2**) [6], 3-(3,4-dimethoxyphenyl)-2-propenal (**3**) [7], and cinnamic acid (**4**) [8], which are reported for the first time from this species. Brahin showed moderate inhibitory activity against the enzyme lipoxygenase.

Brahin (**1**) was obtained as a colorless gummy solid, which gave positive Libermann–Burchardt and $CeSO_4$ tests for a triterpene. The molecular formula was deduced as $C_{36}H_{52}O_8$ by positive mode HR-FAB-MS, which showed a quasimolecular $[M + H]^+$ peak at *m/z* 613.3740 (calcd 613.3744). The IR spectrum showed the presence of hydroxyl (3318 cm^{-1}), ester carbonyl (1725 cm^{-1}), carbonyl (1698 cm^{-1}), and olefinic (1665 cm^{-1}) functionalities. The ^{13}C NMR (BB and DEPT) spectra (Table 1) showed 36 signals comprising six methyl, nine methylene, 12 methine, and 9 quaternary carbons. The downfield signals at δ 209.5 and 175.2 were assigned to the carbonyl and ester moities. The signals at δ 150.1, 145.0, 129.8, 128.3, 115.8, and 109.3 were due to six olefinic carbons. An anomeric carbon was observed at δ 101.4, while the rest of the signals of a sugar moiety resonated in the range δ 77.6–64.4. The presence of the hexose moiety was further confirmed by HR-EI-MS, which showed a daughter fragment at *m/z* 451.3212 $[(M + H) – \text{hexose}]^+$ ($C_{30}H_{43}O_3$). The ^1H NMR spectrum displayed the signals for secondary methyl at δ 1.05 (3H, d, J = 6.6 Hz) and five tertiary methyl signals at δ 1.29, 1.15, 1.10, 1.09, and 0.94 (3H, each s). The olefinic protons resonated as double doublets at 5.28 and as multiplets at δ 5.32 and 5.40 as well as singlets at δ 4.91 and 4.84.

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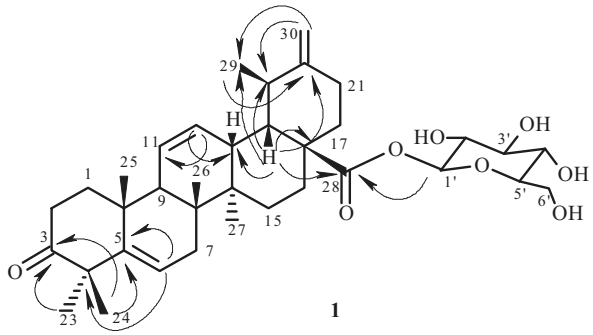
TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1	2.10 (ddd, J = 14.7, 10.5, 7.3);	38.2	18	2.32 (dd, J = 11.1, 5.0)	42.4
	1.92 (ddd, J = 14.7, 6.6, 3.6)		19	2.23 (m)	36.1
2	2.52 (ddd, J = 15.7, 10.5, 7.3);	29.5	20	–	150.1
	2.36 (ddd, J = 15.7, 6.6, 3.6)		21	1.89 (m); 1.25 (m)	32.4
3	–	209.5	22	2.00 (m); 1.66 (m)	33.5
4	–	49.6	23	1.15 (s)	19.3
5	–	145.0	24	1.10 (s)	23.2
6	5.28 (dd, J = 8.0, 3.0)	115.8	25	0.94 (s)	15.8
7	2.04 (dd, J = 15.1, 8.0);	34.1	26	1.09 (s)	12.0
	1.74 (dd, J = 15.1, 3.0)		27	1.29 (s)	24.3
8	–	37.8	28	–	175.2
9	1.64 (m)	54.4	29	1.05 (d, J = 6.6)	13.4
10	–	39.8	30	4.91 (s); 4.84 (s)	109.3
11	5.32 (m)	128.3	1′	4.82 (d, J = 7.1)	101.4
12	5.40 (m)	129.8	2′	3.59 (m)	75.0
13	–	49.8	3′	3.62 (m)	77.2
14	–	40.2	4′	4.10 (m)	71.5
15	2.14 (m); 1.87 (m)	27.4	5′	4.19 (m)	77.6
16	2.20 (m); 1.95 (m)	30.2	6′	4.48 (d, J = 11.5)	64.4
17	–	50.1		4.30 (dd, J = 11.5, 7.0)	



1

Fig. 1. Important HMBC correlations of **1**.

The doublet of methyl protons at δ 1.05 and the double doublets of the C-18 methine proton at δ 2.32 (J = 11.1, 5.0) suggested that compound **1** belongs to the ursene series. The anomeric proton of the sugar moiety was observed as a doublet at δ 4.82 (J = 7.1 Hz), and further signals of oxymethine and oxymethylene protons of the hexose moiety were observed at δ 3.59–4.48. The larger coupling constant of the anomeric proton signal allowed us to assign the *β*-linkage to the hexose moiety. The NMR data confirmed an urs-Δ^{11}-type skeleton for brahin (**1**). The retro-Diels-Alder fragments at *m/z* 164.1201 ($C_{11}H_{16}O$) and 286.1932 ($C_{19}H_{26}O_2$) revealed the presence of the carbonyl moiety in rings A/B and the ester functionality and hexose moiety in rings D/E. Acid hydrolysis provided the residue, which could be identified as D-glucose by the sign of its optical rotation as well as co-TLC with an authentic sample. The structure of brahin (**1**) was confirmed by HMBC correlations (Fig. 1), in which H_3-23 and H_3-24 showed ^{3}J correlations with the carbonyl carbon at δ 209.5 and olefinic carbon at δ 145.0. The proton H-18 showed ^{2}J correlation with C-17 (δ 50.1), C-19 (δ 36.1), and C-13 (δ 49.8), as well as ^{3}J correlations with ester carbonyl (δ 175.2), C-20 (δ 150.1), and C-29 (δ 13.4). The olefinic proton at C-11 (δ 5.32) showed ^{2}J connectivities with C-9 (δ 54.4) and C-12 (δ 129.8), as well as ^{3}J correlations with C-8 (δ 37.8), C-10 (δ 39.8), and C-13 (δ 49.8). The anomeric proton at δ 4.82 showed ^{3}J correlations with the ester carbonyl carbon at δ 175.2. On the basis of these evidences, brahin (**1**) could be assigned the structure 3-oxo-5,11,20(30)-ursatriene-17-(*β*-D-glucopyranosyl) ester.

Lipoxygenase (LOX, EC 1.13.11.12) is a key enzyme in the biosynthesis of a variety of bioregulatory compounds such as hydroxyeicosatetraenoic acids (HETEs), leukotrienes, lipoxins, and hepoxylines. It has been found that LOX plays a role in a variety of disorders such as bronchial asthma, inflammation, and tumor angiogenesis [9]. Compound **1** showed moderate inhibitory activity against lipoxygenase with an IC_{50} value of 56.75 μM, compared with the IC_{50} value of 8.01 μM observed for baicalein used as positive control.

EXPERIMENTAL

General Precedure. Optical rotations were recorded on a JASCO DIP-360 digital polarimeter. IR spectra were recorded on a JASCO 302-A spectrophotometer in KBr. NMR spectra were measured on a Bruker 500 MHz instrument. Chemical shifts δ are expressed in ppm, and coupling constants J are expressed in Hz. EI and HR-FAB-MS were recorded on JEOL JMS-HX-110 and JMS-DA-500 mass spectrometers using glycerol as matrix. Silica gel (250–400 mesh; E. Merck, Darmstadt, Germany) was used for column chromatography (CC); silica-gel 60 F_{254} plates (E. Merck, Darmstadt, Germany) were used for TLC.

Plant Material. The whole plant of *S. brahuica* Boiss. (10 kg) was collected from Ziarat Valley, Balochistan region and identified by plant taxonomist Prof. Dr. Rasool Bakhsh Tareen, Department of Botany, University of Balochistan, Pakistan where a voucher specimen (No. SB.R.B.T.08.BUH) has been deposited in its Herbarium.

Extraction and Isolation. *S. brahuica* (10 kg) was shade-dried, ground, and extracted with ethanol (3 × 40 L). The combined ethanolic extract was freed of solvent to obtain a crude residue (450 g), which was divided into fractions soluble in *n*-hexane (70 g), $CHCl_3$ (40 g), EtOAc (12 g), *n*-BuOH (250 g), and H_2O (40 g). The $CHCl_3$ soluble fraction (40 g) was subjected to column chromatography (CC) eluting with mixtures of *n*-hexane–EtOAc in incresing order of polarity to furnish three major fractions A–C. Fraction A eluted with *n*-hexane–EtOAc (7:3) was further chromatographed and eluted with *n*-hexane–EtOAc (8:2) to obtain two successive fractions A_1 and A_2. Column chromatography of fraction A_1 and elution with *n*-hexane–EtOAc (9:1) provided brahin (**1**) (9 mg). The CC of fraction A_2 and elution with *n*-hexane–EtOAc (7.5:2.5) afforded *β*-sitosterol (**2**) (27 mg). Fraction B eluted with *n*-hexane–EtOAc (4:6) furnished cinnamic acid (**4**) (15 mg). Fraction C eluted with *n*-hexane–EtOAc (2:8) was re-chromatographed using *n*-hexane–EtOAc (4:6) as eluent to obtain two successive fractions C_1 and C_2. The CC of subfraction C_2 eluted with *n*-hexane–EtOAc (4:6) afforded 3-(3,4-dimethoxyphenyl)-2-propenal (**3**) (10 mg).

Brahin (1). Colorless gummy solid; $[\alpha]_D^{20}$ +35.2° (*c* 0.02, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3318, 1725, 1698, 1665. EI-MS (*m/z*, $I_{rel.}$, %): 451 [M – hexose + H^+, 9], 286 (25), 164 (100), 162 (55). HR-FAB-MS (pos. mode) *m/z* 613.3740 $[M + H]^+$ (calcd for $C_{36}H_{53}O_8$, 613.3744). For ^1H (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1.

Acid Hydrolysis of Compound 1. Compound **1** (5 mg) was dissolved in MeOH (6 mL) containing 1 N HCl (3 mL) and refluxed for 4 h. It was concentrated under reduced pressure and diluted with water and extracted with EtOAc. The aqueous phase was concentrated to obtain the residue, which was identified as D-glucose by the sign of its optical rotation $[\alpha]_D^{23}$ +51.3° (*c* 0.02 MeOH) and as well as co-TLC with an authentic sample. The aglycone could not be obtained due to the paucity of material.

***In vitro* Lipoxygenase Inhibitory Assay**. Lipoxygenase inhibitory activity was determined by slightly modifying the spectrometric method developed by Tappel [10]. Lipoxygenase (1.13.11.12) type I-B (from soybean) and linoleic acid were purchased from Sigma Chemicals. A mixture of 160 mL of 100 mM phosphate buffer (pH 5.0), 10 mL of test compound, and 20 mL of lipoxygenase solution was incubated for 10 min at 258 nm. The reaction was then initiated by the addition of 10 mL linoleic acid (substrate) solution [11], resulting in the formation of (9*Z*,11*E*,13*S*)-13-hydroperoxyoctadeca-9,11-dienoate. The change in absorbance was followed for 6 min at 234 nm. The test compounds and the control were dissolved in MeOH or 50% EtOH. All the reactions were performed in triplicate on a 96-well plate reader Spectromax 384 plus (Molecular Devices, USA). The IC_{50} values were calculated using the EZ-Fit Enzyme Kinetics Program (Perrella Scientific Inc., Amherst, USA). The percentage (%) inhibition was calculated by the formula (E–S)/E × 100, where E is the activity of the enzyme without test compound and *S* is the activity of the enzyme with test compound.

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