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PAASHAANOLACTONE FROM *BERGENIA LIGULATA*

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Key Word Index—*Bergenia ligulata*; Saxifragaceae; paashaanolactone; paashaanbhed.

Abstract—Four compounds have been isolated from the *Bergenia ligulata* rhizomes, among which one is new 4(4′-β-D-glucopyranosyloxy-1′-benzoyloxy)-6-methyltetrahydropyran-2-one named as paashaanolactone (**1**) and the structure was established by detailed spectroscopic studies.

INTRODUCTION

Bergenia ligulata Yeo., is a medicinal plant distributed in South and East Asia. In India it grows at high altitudes in the Himalayas usually in rocky areas and cliffs. It is popularly known in the Indian system of medicine as Paashaanbhed. The rhizomes have been used for centuries in the Ayurvedic formulations for various ailments [1]. Alcoholic extract of the plant has exhibited significant analgesic, anti-inflammatory and diuretic properties [2]. Previous chemical investigations of the plant have indicated the presence of β-sitosterol, β-sitosterol-D-glucoside, bergenin [3] and afzelechin [4]. A preliminary screening showed that the methanolic extract of *Bergenia ligulata* possesses significant anti-inflammatory activity (58%) in carrageenan induced paw oedema model in albino rats. This prompted us to undertake an investigation of the constituents of this plant. The phytochemical investigation resulted in the isolation of four compounds among which one is a new compound. This paper principally deals with the isolation and structure elucidation of the new compound named by us as paashaanolactone (**1**) by a combination of spectroscopic methods.

RESULTS AND DISCUSSION

The FAB-mass spectrum of compound **1** shows a $[M+H]^+$ peak at *m/z* 413 consistent with the formula $C_{19}H_{24}O_{10}$ which was confirmed by ^{13}C NMR and DEPT experiments and an adduct ion at *m/z* 435 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra (Table 1) showed signals attributed to one glucose moiety. The coupling constant ($J = 7.7$ Hz) of the signal resulting from the anomeric proton of the glucopyranoside indicated the glucosidic linkage to have β-configuration. The ^1H NMR spectra showed the two coupled doublets (2H each) at δ 6.86 and 7.88 ($J = 8.5$ Hz) indicated the existence of a *p*-hydroxybenzoyl group. The aromatic region of COSY spectrum reveals only the coupling of the AA′BB′ system of *p*-hydroxybenzoyl moiety. This moiety was also deduced from the FAB-mass spectral data which shows a fragment at *m/z* 121 owing to the loss of the *p*-hydroxybenzoyl group. This conclusion was also confirmed by the ^{13}C NMR signals (δ_C 165.8, 161.6, 131.2, 120.4 and 114.9). Furthermore, the ^1H NMR and ^1H-^{13}C HETCOR spectra contained a methyl doublet signal at δ 1.24 ($J = 6.3$ Hz), δ_C 20.7, two oxygen bonded methine proton signals at δ 4.78 *m*, δ_C 72.4 and δ 4.21 *m*, δ_C 71.1; methylene protons at δ 1.56 *dd* ($J = 2.7$, 11.4 Hz), 2.25 *m*, δ_C 35.3 and 2.60 *dd* ($J = 5.1$, 17.9 Hz), 2.80 *m*, δ_C 35.6 and signal of a lactone carbonyl at δ 169.7. ^1H-^1H COSY indicated the interaction of 6-H with methyl, 5a-H and 5b-H protons. Furthermore, 4-H showed the interaction with 3a-H, 3b-H, 5a-H and 5b-H protons. Thus, based on these data part structure of **1** was established as δ-lactone with oxygen substituent at C-4 and methyl at C-6. Since C-4H and C-6H are both attached to acyl function the signals are expected to be downfield compared to their respective position if attached to oxygen function without being acylated. To put them in proper place, one signal being at δ 4.21 (1H) compared to other appearing at 4.78 (1H) clearly indicates that former is axial and latter is equatorial. Furthermore,

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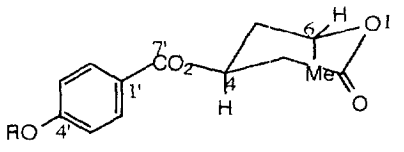
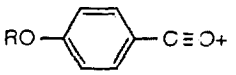


1: R = β-D-glucopyranosyl
2: R = tetra-O-acetyl-β-D-glucopyranosyl



3: m/z = 283: R = β-D-glucopyranosyl
4: m/z = 451: R = tetra-O-acetyl-β-D-glucopyranosyl

the C-4 proton signal shows sufficient width at half-height ($W_{h/2}$ 10.0 Hz in **1** and 9.10 Hz in **2**) supports the proposed axial arrangement. In support to these assignments COSY experiment were conducted which clearly indicated the position of C-4H and C-6H. From the molecular model it is clear in a chair form of δ-pyrone that the preferred conformation should favour the bulky benzoate into equatorial position and from this assumption the C-4H has to take axial position and C-6H equatorial, which is supported by their chemical shifts. Furthermore, compound **1** on acid hydrolysis furnished glucose and *p*-hydroxybenzoic acid, identified by comparison with authentic samples (tlc).

Compound **1** on acetylation gave tetra-acetate **2**, $C_{27}H_{32}O_{14}$(FAB-mass spectrum *m/z* 581 $[M+H]^+$, 603 $[M+Na]^+$). The ^1H, ^1H-^1H COSY, ^1H-^{13}C HETCOR, DEPT experiments indicated the presence of tetra-acetylglucose, *p*-hydroxybenzoyl and δ-lactone units. The sugar moiety is linked with the phenolic OH because the chemical shift of the sugar binding proton and carbon is similar to that of *p*-β-D-glucosyloxy benzoic acid [5, 6]. This is further supported by fragment **3** at *m/z* 283 (60%) in compound **1** and fragment **4** at *m/z* 451 (100%) in compound **2** in the FAB-mass spectra, which were formed by cleavage at the ester bond [7]. Thus, the glucose moiety is linked with the phenolic OH and the δ-lactone at the carboxylic group.

Thus, the above analysis led to the structure 4(4′-β-*d*-glucopyranosyloxy-1′-benzoyloxy)-6-methyltetrahydropyran-2-one (**1**), which we have given name the paashaanolactone, reported for the first time from this plant source.

Table 1. ^1H and ^{13}C NMR data of compounds **1** and **2**

Position	**1*** δ_H (*J* in Hz)	**1*** δ_C	**2**$^+$ δ_H (*J* in Hz)	**2**$^+$ δ_C
1	—	—	—	—
2	—	169.7	—	169.4
3	2.62 *dd* (5.1, 17.9)	35.6	2.58 *dd* (4.5, 17.7)	35.2
	2.80 *m*		2.65 *m*	
4	4.21 *m*	71.1	4.27 *m*	70.5
5	1.56 *dd* (2.7, 11.4)	35.5	1.65 *dd* (2.8, 11.5)	35.9
	2.25 *m*		2.18 *m*	
6	4.78 *m*	72.4	4.70 *m*	72.8
7	1.24 *d* (6.3)	20.7	1.33 *d* (6.4)	21.4
1′	—	120.4	—	127.0
2′, 6′	7.88 *d* (8.5)	131.2	8.08 *d* (8.6)	131.3
3′, 5′	6.86 *d* (8.5)	114.9	7.20 *d* (8.6)	121.9
4′	—	161.6	—	154.7
7′	—	165.8	—	165.3
1″	4.38 *d* (7.7)	102.5	4.64 *d* (8.0)	99.3
2″	3.28 *t* (8.1)	72.9	4.98 *t* (8.0)	71.0
3″	3.45 *m*	76.3	5.16 *t* (9.5)	72.5
4″	3.45 *m*	69.9	5.25 *t* (9.4)	68.5
5″	3.59 *m*	73.7	3.85 *m*	72.0
6″	4.41 *dd* (6.4, 12.0)	63.2	4.35 *dd* (4.9, 12.3)	62.5
	4.62 *dd* (2.0, 12.0)		4.56 *dd* (2.4, 12.3)	
OCOMe			2.04 *s*	21.2
			2.03 *s*	20.6
			2.02 *s*	
OCOMe				170.2
				169.4
				169.1
				168.9

*In DMSO-d_6 + $CDCl_3$.
$^+$In $CDCl_3$.

The three known compounds (+)-catechin, (+)-catechin-7-O-β-D-glucopyranoside [8] and 11-*O*-galloyl bergenin [9] were isolated for the first time from this plant and identified based on their NMR spectral data, and by comparison of their physical properties with those reported in the literature.

EXPERIMENTAL

General procedure. Mps: uncorr., IR: KBr, OR were measured on Perkin–Elmer model 241 polarimeter using a Na lamp. The ^{1}H and ^{13}C NMR at 300 MHz and 75 MHz, respectively. Flash chromatography was carried out using silica gel (230–400 mesh) and TLC over E. Merck precoated silica gel plates. Visualization was by UV (254 nm) and by spraying with acidified vanillin followed by charring.

Plant materials. The plant material was collected from Moussauri hills of U. P. India and was identified as *Bergenia ligulata* by Dr. Y. K. Sarin, Department of Botany, Regional Research Laboratory, Jammu 180 001, India and voucher specimen (997 and 998) was deposited in the herbarium of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160 014, India.

Extraction and isolation. The powdered rhizomes of *Bergenia ligulata* (1 kg) were successively percolated with hexane, $CHCl_3$ and MeOH. The extracts were screened for anti-inflammatory activity using carrageenan induced paw oedema model in Wistar albino rats. The most promising activity was observed in the MeOH extract (58%), compared with standard ibuprofen. The MeOH extract on repeated flash chromatography over silica gel allowed the isolation of paashaanolactone (**1**), (+)-catechin, (+)-catechin-7-O-β-D-glucopyranoside and 11-O-galloyl bergenin.

Paashaanolactone (**1**). Recrystallized from MeOH, mp 116–118°, $[\alpha]_D^{25}$ +11.33° (MeOH, *c* 3.53); *IR* ν_{max}^{KBr} cm^{-1}: 3324, 1705, 1676, 1606, 1594, 1272; FAB-MS *m/z*, 435 $[M+Na]^+$, 413 $[M+H]^+$, 283, 176, 137, 121, 107, 93, 65, 39; for ^{1}H- and ^{13}C- NMR spectral data see Table 1.

Paashaanolactone tetra-acetate (**2**). Recrystallized from $CHCl_3$, mp 164–165°, $[\alpha]_D^{25}$ +6.45° ($CHCl_3$, *c* 2.45); IR ν_{max}^{KBr} cm^{-1}: 1734, 1604, 1368, 1196; FAB-MS *m/z* 603 $[M+Na]^+$, 581 $[M+H]^+$, 451, 331, 137, 121, 113, 69, 43; for ^{1}H and ^{13}C NMR spectral data see Table 1.

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