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MACROPHYLLOSIDE, A FLAVONE GLUCOSIDE FROM *PRIMULA MACROPHYLLA*

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Key Word Index—*Primula macrophylla*; Primulaceae; flavone glucoside; macrophylloside; 2'-hydroxy-7-*O*-β-D-glucopyranosyloxyflavone.

Abstract—A new flavone glucoside macrophylloside has been isolated from the whole plant of *Primula macrophylla* and its structure was determined by spectroscopic methods as 2'-hydroxy-7-*O*-β-D-glucopyranosyloxyflavone. Sitosterol glucoside was also isolated for the first time from this plant.

INTRODUCTION

Primula macrophylla D. Don. (syn. *P. stuartii* Wall.) is a perennial herb found on K-2 in the Karakorum and Kuram Valley in Pakistan at altitudes of 12000–16000 ft [1]. The plant was collected from 'Sora Lusht' (16000 ft) near the Pakistan–Afghanistan boarder. The leaf exudate is used locally for the treatment of eye diseases. A number of unusual flavones have been reported from the leaf exudate of *Primula* species [2] but this plant has not been chemically investigated so far. Our phytochemical investigation of *Primula macrophylla* has resulted in the isolation and structure elucidation of a new flavone glucoside.

RESULTS AND DISCUSSION

Macrophylloside (**1**) mp 66–67° was isolated as a crystalline solid from the methanolic soluble portion of the whole plant as described in the Experimental. The FAB mass spectrum of **1** (negative ion mode) shows pseudo-molecular ion peak at *m/z* 415 $[M-1]^-$ and fragments at *m/z* 253 $[M-H-162]^-$ indicating the loss of hexosyl moiety, *m/z* 297 and *m/z* 117. The positive ion FAB high resolution mass spectrum shows $[M+1]^+$ peak at 417.1193 (calcd. for $C_{21}H_{21}O_9$, 417.1179) which corresponds to molecular formula $C_{21}H_{20}O_9$ for **1**. The UV spectrum displayed maxima at 243, 212 (sh) and 315 nm, indicating **1** to be a flavonoid [3]. The appearances of these bands at shorter wave lengths indicate the presence of glucosyl group [4] and the absence of a OH-5 group [5] in **1**. No large bathochromic shift was observed in the UV spectrum with NaOMe, indicating the absence of a OH-4' group [5]. Compound **1** was light blue in UV light and turns to yellowish green after exposure to NH_3 vapours which indicates lack of 5-*O*-glucoside group [4]. Its IR spectrum contains peaks at 3200 cm^{-1} (OH), 1610 cm^{-1} (conjugated carbonyl group) and at 1550–1500 cm^{-1} (aromatic ring).

The ^1H NMR spectrum showed signals at δ6.89 (1H, *s*, H-3), 7.27 (1H, *dd*, *J* = 0.8, 6.3 Hz, H-5), 7.55 (1H, *dd*, *J*

Table 2. ^1H NMR spectral data of compound **1** and its penta-acetate **2** (400, 300 MHz, CD_3OD)

H	1	2
3	6.81 *s*	6.88 *s*
5	7.34 *dd* (0.8, 8.0)	7.36 *br d* (8.7)
6	7.53 *dd* (2.0, 8.0)	7.72 *dd* (1.5, 8.7)
8	7.52 *s*	7.82 *d* (2.1)
3'	7.70 *dd* (1.2, 8.4)	7.96 *dd* (2.4, 8.7)
4'	7.81 *ddd* (1.6, 7.2, 8.8)	7.81 *ddd* (1.8, 7.2, 8.4)
5'	7.49 *ddd* (0.8, 8.0, 8.0)	7.50 *ddd* (1.2, 7.2, 8.1)
6'	8.14 *dd* (1.6, 7.6)	8.14 *dd* (1.6, 7.6)

Table 1. Results of homonuclear decoupling experiments

No.	Signal irradiated δ (H)	Results δ (H)
1	7.27 (H-5)	*dd* at 7.55 (H-6)→*d* (*J* = 2.7 Hz)
2	7.55 (H-6)	*dd* at 7.27 (H-5)→*br s*
3	7.77 (H-3')	*ddd* at 7.82 (H-4')→*dd* (*J* = 3.0, 6.9 Hz)
4	7.82 (H-4')	*dd* at 7.77 (H-3')→*s* *ddd* at 7.48 (H-5')→*dd* (*J* = 2.2, 7.2 Hz)
5	7.48 (H-5')	*ddd* at 7.82 (H-4')→*dd* (*J* = 3.0, 8.7 Hz) *dd* at 8.04 (H-6')→*d* (*J* = 2.7 Hz)
6	8.04 (H-6')	*ddd* at 7.48 (H-5')→*dd* (*J* = 2.3, 6.6 Hz)

Table 3. ^{13}C NMR and heteroCOSY of compound 1

C			^1H–^{13}C correlation	C			^1H–^{13}C correlation
2	155.7	C	No coupling	1″	101.3	CH	4.90 (H-1″)
3	105.9	CH	6.89 (H-3)	2″	73.3	CH	3.30 (H-2″)
4	177.0	C	No coupling	3″	75.9	CH	3.38 (H-3″)
5	116.1	CH	7.27 (H-5)	4″	69.8	CH	3.20 (H-4″)
6	118.4	CH	7.55 (H-6)	5″	77.3	CH	3.40 (H-5″)
7	162.6	C	No coupling	6″	60.7	CH_2	3.74, 3.50 (H-6″)
8	113.6	CH	7.53 (H-8)				
9	147.0	C	No coupling				
10	125.2	C	No coupling				
1′	123.3	C	No coupling				
2′	148.4	C	No coupling				
3′	118.4	CH	7.77 (H-3′)				
4′	134.2	CH	7.82 (H-4′)				
5′	125.4	CH	7.48 (H-5′)				
6′	124.8	CH	8.04 (H-6′)				

= 2.4, 6.3 Hz, H-6), 7.53 (1H, *s*, H-8), 7.77 (1H, *dd*, $J = 1.2$, 8.4 Hz, H-3′), 7.82 (1H, *ddd*, $J = 1.5$, 6.6, 8.4 Hz, H-4′), 7.48 (1H, *ddd*, $J = 1.5$, 6.6, 7.8 Hz, H-5′) and 8.04 (1H, *dd*, $J = 1.5$, 7.8 Hz, H-6′). The anomeric proton resonate at δ4.90 (1H, *d*, $J = 7.2$ Hz, H-1″), which proves the site of glucosylation at C-7 position [5], the flavonoid nature of the compound and the β linkage of sugar moiety [6]. The other sugar protons resonate at δ3.30 (1H, overlapping, H-2″), 3.38 (1H, *m*, H-3″), 3.20 (1H, *m*, H-4″), 3.40 (1H, *m*, H-5″), 3.74 (1H, *dd*, $J = 1.8$, 11.7 Hz, H-6″a) and 3.5 (1H, *dd*, $J = 5.7$, 12.0 Hz, H-6″b). The homo-nuclear decoupling experiments and their results are summarized in Table 1. The ^1H NMR assignments were further confirmed with the help of COSY-45, NOESY and 2D-*J*-resolved experiments.

Acetylation of **1** gave the pentaacetate **2** as an amorphous solid. In the ^1H NMR (Table 2), there is a peak at δ2.32 (*s*, 3H) showing aromatic acetyl group at position 2′, alongwith four more peaks resonating at δ2.07, 2.06, 2.04 and 2.00 (*s*, each 3H) for glucosyl acetate. The ^1H NMR of **1** and **2** showed that the H-3′ and H-5′ signals were shifted downfield by 0.26 ppm and 0.01 respectively which showed that H-3′ is *ortho* and H-5′ is *para* to the hydroxyl group. The H-6 and H-8 protons were shifted by 0.19 ppm and 0.30 ppm respectively which confirmed that both of the protons have an *ortho* position to the glucosyl group [6].

The ^{13}C NMR assignments were carried out with the help of DEPT and heteroCOSY experiments. The typical signals (Table 3) of C-2, C-3 and C-4 showed the flavonoid nature of the compound. The downfield shift of C-3 and the upfield shift of C-2 resulted from the hydroxyl group at position C-2′ [7]. The chemical shift of C-7 supported the attachment of glucosyl group at this position [7].

The acidic hydrolysis of **1** yielded the aglycone **3** as a yellow amorphous solid alongwith glucose. The aglycone showed EI mass spectral data $[M]^+$ at *m/z* 254 consistent with the formula $C_{15}H_{10}O_4$ and the fragments at *m/z* 237 $[M-17]^+$, *m/z* 226 $[M-28]^+$, *m/z* 121 $[M-133]^+$ and *m/z* 92 $[M-121-41]^+$. The fragment $[M-17]^+$ at *m/z* 237 by the loss of hydroxyl group further prove the presence of hydroxyl group at the C-2′ position [3, 4]. The UV spectrum displayed absorption maxima at 276 and 337 nm, confirming that **1** is a glucoside of **3**. With NaOMe there is a band at 318 nm, confirming the hydroxyl group at C-7 position [4] which further support the presence of glucosyl group at this position. The sugar moiety which was identified as D-glucose through co-TLC and co-PC with an authentic sample. It was confirmed to be *O*-β-D-glucose by comparison of its ^{13}C NMR spectral data (Table 3) with that in the literature [7]. The mass fragmentation pattern of the FAB and EI mass spectra also supported the assignment of the *O*-glucosyl and the hydroxyl group attached to the ring A and B respectively.

EXPERIMENTAL

Extraction and isolation. The dried plant (*ca* 15 kg) of *Primula macrophylla* D. Don. was extracted with MeOH. The crude extract was evapd under red. pres. at 60° and fractionated with EtOAc–H_2O (1:1). The aq. layer was partitioned with *n*-butanol–H_2O (1:1). The *n*-butanol layer was evapd under red. pres. at 70°. The residue obtained was dissolved in a min. quantity of MeOH and Et_2O was added dropwise. The resulting ppt. contained polar compounds. It was dissolved in MeOH and chromatographed on a column with silica gel (70–230 mesh). Elution with $CHCl_3$–MeOH (9:1) afforded **1** which was obtained as a crystalline solid, mp 66–67°.

Sitosterol glucoside was obtained in $CHCl_3$–MeOH (9:1) and crystallized in MeOH, mp 280°. The NMR data in CD_3OD was compared with the data given in the literature. The sugar moiety was compared with an authentic sample of D-glucose after hydrolysis.

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