DOI 10.1007/s10600-015-1311-y
Chemistry of Natural Compounds, Vol. 51, No. 3, May, 2015

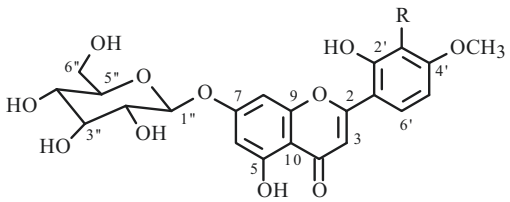
ISOLATION AND CHARACTERIZATION OF A NEW FLAVONOID GLUCOSIDE FROM AERIAL PARTS OF *Phrynium placentarium*

U. Ch. De,[1*] J. Bhowmik,[1] S. Chowdhury,[2] A. Basak,[3] and B. Dinda[1]

*A new flavonoid glucoside (**1**) has been isolated from the aerial parts of* Phrynium placentarium *(Marantaceae). The structure of **1** was established as 5,2′-dihydroxy-3′,4′-dimethoxyflavone-7-*O*-β-*D*-glucopyranoside by means of chemical and spectral data including 2D NMR studies. Compound **1** was examined for antioxidant and PCSK3 (furin) inhibition efficacy because inhibitors of furin are under consideration as therapeutic agents for treating various diseases, including HIV, influenza, dengue fever, etc. Experimental results showed antioxidant activity with EC_{50} value of 100 μg/mL and PCSK3 inhibition activity with EC_{50} value of 80 μg/mL on spectrophometric assays.*

Keywords: *Phrynium placentarium*, Marantaceae, flavonoid glucoside.

Phrynium placentarium (Marantaceae) is 1–2 m tall, having basal leaf and flowers two per bract; it is white to yellowish white and grows in wet shaded places in forest lands of Bhutan, Indonesia, Myanmar, Philippines, Thailand, Vietnam, and the northeastern part of India [1]. It is used as a traditional medicine of the local tribes for the treatment of renal disorder [2, 3] but has not been phytochemically studied. We have investigated the aerial parts of this plant for the first time and isolated a light yellow colored compound **1**. Compound **1** was characterized by chemical and spectroscopic (NMR and mass) methods and identified as a flavonoid glucoside. To the best of our knowledge, compound **1** is a new natural product. As part of our regular work, compound **1** was examined for its antioxidant as well as furin inhibition activity. This article reports the isolation and structure elucidation of **1** from the aerial parts of *P. placentarium* and its efficacy as an antioxidant as well as PCSK3 (furin) inhibitor.



1, 2

1: R = OCH_3

2: R = H

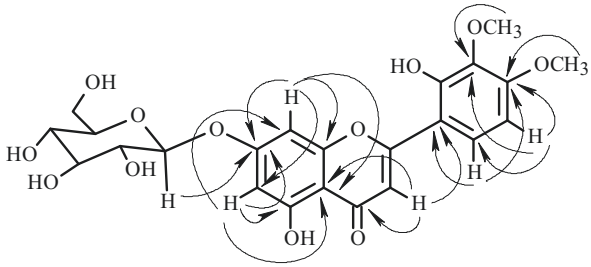
Compound **1** was isolated as yellowish needles from the methanolic extract of aerial parts of *P. placentarium* via repeated column chromatography and was assigned the molecular formula $C_{23}H_{24}O_{12}$ by HR-FAB-MS (+) 493.6039 $[M + H]^+$ (calcd for $[M]^+$ 492.6013). The UV spectrum showed λ_{max} at 279 and 350 nm, indicating the presence of the flavonoid skeleton in **1**. The IR spectrum showed broad absorption at 3612 cm^{-1} (OH stretching) along with absorption bands at 1650 and 1600 cm^{-1} (characteristic of chromone carbonyl), also suggesting the flavonoid-like structure of **1**.

1) Department of Chemistry, Tripura University, Suryamaninagar-799 022, Agartala, Tripura, India, e-mail: ucd1972@gmail.com; 2) Department of Chemistry, R. K. Mahavidyalaya, 799277, Kailasahar, Tripura, India; 3) Chronic Disease Program, Regional Protein Chemistry Center, Ottawa Hospital Research Institute, U Ottawa, 725 Parkdale Avenue, Ottawa, ON K1Y 4E9, Canada. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2015, pp. 389–391. Original article submitted September 14, 2013.

TABLE 1. ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data of compounds **1** and **2** (δ, ppm, J/Hz)

C atom	**1** (DMSO-d_6)		**2** ($CDCl_3$)	
	δ_H	δ_C*	δ_H	δ_C
2	–	161.12 (C)	–	161.16
3	6.98	103.79 (CH)	6.79 s	103.20
4	–	182.11 (C)	–	181.93
5	12.98 (s, OH)	164.22 (C)	12.9 (OH)	164.50
6	6.46 (d, J = 1.2)	99.54 (CH)	6.46 (d, J = 1.8)	99.57
7	–	163.12 (C)	–	162.98
8	6.87 (d, J = 1.2)	95.35 (CH)	6.76 (d, J = 1.8)	97.76
9	–	156.97 (C)	–	156.98
10	–	105.38 (C)	–	105.37
1′	–	121.29 (C)	–	121.42
2′	9.36 (s, OH)	151.07 (C)	9.5 (OH)	149.95
3′	–	148.27 (C)	7.12 (d, J = 3.4)	113.59
4′	–	148.12 (C)	–	145.81
5′	6.95 (d, J = 9)	115.38 (CH)	7.44 (dd, J = 3.4, 8.4)	116.02
6′	7.60 (d, J = 9)	120.57 (CH)	6.92 (d, J = 8.4)	119.21
2 × OCH_3	3.90 s, 3.88 s	56.40, 56.0 (OCH_3)	3.61 s	55.82
1″	5.06 (d, J = 7.2)	100.02 (CH)	5.09 (d, J = 7.2)	99.92
2″		69.67 (CH)	3.29 (d, J = 8.8)	70.36
3″	3.18–3.46 m	73.19 (CH)	3.34 (t, J = 8.8)	73.15
4″		76.48 (CH)	3.32 m	76.42
5″		77.39 (CH)	3.49 m	77.19
6″	3.73 (dd, J = 8.4, 4.2)	60.67 (CH_2)	3.73 m	60.65
Glc OH	5.43, 5.16, 5.09, 4.66 (each br.s, disappeared in D_2O)	–	–	–

*Assignment based on 2D HSQC and HMBC.



1

Fig. 1. ^{1}H–^{13}C HMBC Correlations of **1**.

The ^{13}C NMR (DEPT) (Table 1) showed 23 carbon signals, *viz*. two methyls, one methylene, 10 methines and 10 quaternary carbons, which was further supported by the correlated carbon signals from the ^{1}H–^{13}C HSQC spectrum of the compound. Of these, 15 signals were attributed to the flavone skeleton and six to a hexose sugar unit, while the remaining two were attributed to two methyl carbons. ^{1}H NMR resonances (Table 1) at δ 5.06 (1H, d, J = 7.2 Hz) and 3.18–3.73 ppm also indicated the presence of a sugar unit. The sugar was identified as D-glucose by hydrolysis and direct comparison [co-TLC, PC and $[\alpha]_D$ +52.6° (*c* 0.11, H_2O)] with an authentic sample. The $^{3}J_{H\text{-}1''/H\text{-}2''}$ coupling constant (diaxial coupling) of the anomeric proton corroborated the *β*-linking nature of the sugar unit with the aglycone in compound **1** [4]. The upfield chemical shift of C-7 carbon (δ 163.12) along with the correlation of H-1″ with C-7 in HMBC (Fig. 1) experiments confirmed the linkage of the glucose unit to the aglycone via the C-7 hydroxy group [5]. The ^{1}H NMR signals at δ 6.46 (1H, d, J = 1.2 Hz) and 6.87 (1H, d, J = 1.2 Hz) ppm indicated the presence of *meta*-coupled aromatic protons, while the signals at δ 6.95 (1H, d, J = 9 Hz) and 7.60 (1H, d, J = 9 Hz) ppm indicated the presence of *ortho*-coupled aromatic protons. A detailed analysis of the HMBC and COSY experiments led us to consider that the first pair was due to the proton spin system of C-6 and C-8, while the other pair was due to C-5′ and C-6′. The ^{1}H NMR spectrum also exhibited two broad singlets at δ_H 12.98 and 9.36 ppm (vanishing on D_2O exchange), which indicated the presence of two hydroxyl groups in which the former was a characteristic

proton signal of a chelated hydroxyl and was assigned to 5-OH of the flavonoidic aglycone. The HMBC of H-5′ with C-3′, C-4′, and C-6′ and H-6′ with C-1′, C-4′, and C-5′ unambiguously confirmed the position of the other hydroxyl at C-2′ of the B ring [6–8]. Two sharp singlets in the ^{1}H NMR at δ 3.88 and 3.90 ppm integrated for six protons were assigned to two OCH_3 groups. Analysis of the correlation spectroscopy (COSY and HMBC) placed the two OCH_3 groups at C-3′ and C-4′ of the B ring in compound **1** [8]. Based on the foregoing analyses and comparison of the spectral data with those of compound **2** isolated from *Salix denticulate* [9], the structure of compound **1** was established as 5,2′-dihydroxy-3′,4′-dimethoxyflavone-7-*O*-*β*-D-glucopyranoside. The small variation between the NMR data of **1** and **2** (Table 1) was possibly due to different substitution patterns in the B ring and the solvent effect.

Compound **1** was examined for antioxidant and furin (proprotein convertase subtilisin/kexin 3) inhibition efficacy because inhibitors of furin (PCSK3) are under consideration as therapeutic agents for treating various diseases, including cancer, HIV, influenza, dengue fever, etc. [10]. Proprotein convertases (PCs) or proprotein convertase subtilisin/kexins (PCSKs) belong to a family of calcium-dependent endoproteases that are structurally related to bacterial subtilisin and yeast kexin. These enzymes play major roles in the processing of inactive precursor proteins producing their bioactive mature forms that are implicated in a wide variety of diseases, including cancer and viral and bacterial infections. These events take place in a highly selective, orchestrated, and stepwise manner. Among the various proprotein substrates of PCSK enzymes, particularly important are the precursor growth factors that include different growth factors like proIGF-1, 2 and proVEGF-C because of their strong implications in cancer initiation, progression, and metastasis. As a result of these findings, PCSK enzymes, particularly furin or PCSK3, became a major target for possible interventions in the aforesaid diseases via the use of selective inhibitors [10, 11]. Significant progress has been accomplished in the development of peptide and protein-based inhibitors. However, nonpeptide inhibitors are preferred because of their druglike and other characteristics. So far, very few flavonoid inhibitors for PCSK3 enzymes have been described in the literature [12]. On the other hand, flavonoids have remarkable antioxidant properties and different plant sources are extensively investigated for their potential antioxidant activity [13]. In this context compound **1** was examined for furin inhibition as well as for antioxidant activity by *in vitro* spectrophotometric methods. Experimental results showed that compound **1** has considerable activity with EC_{50} values of 100 μg/mL and 80 μg/mL as antioxidant and as PCSK3 (furin) inhibitor, respectively.

EXPERIMENTAL

General. IR, PerkinElmer FTIR-100 spectrometer; UV, PerkinElmer Lamda 25; $[\alpha]_D$, PerkinElmer 241polarimeter; NMR, Brucker 600 spectrometer; HR-FAB-MS and FAB-MS, Jeol JMS-HX 110 mass spectrometer. Diaion HP-20 (Sigma Aldrich, India) and silica gel (60–120 mesh, Merck, India) were used for CC, and silica gel G (Merck, India) for TLC. Recombinant-soluble furin enzyme (New England Bio Labs Inc., USA), fluorogenic substrate Boc-RVRR-MCA (Louisville, KY, USA), and a spectrofluorometer (Gemini, Molecular Devices, Sunnydale, CA, USA) were used for furin inhibition assay. DPPH and BHT (Sigma Aldrich, India) were used for antioxidant activity study.

Plant Material. Aerial parts of the plant were collected from Amarpur, South Tripura District in July, 2010. The plant was independently identified by Dr. N. K. Chakraborty, Retired Professor of Botany, M. B. B. College, Agartala and by Dr. B. K. Datta, Professor, Department of Botany, Tripura University. A voucher specimen (TU/H/1326) has been deposited at the Herbarium of Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura University, Agartala, India.

Extraction and Isolation. Fresh air-dried ground aerial parts (3 kg) were extracted with MeOH (4 L × 2). The concentrated semisolid extract (285 g) was suspended in H_2O (ca. 250 mL), defatted with hexane, and then successively partitioned with $CHCl_3$, EtOAc, and *n*-butanol. The *n*-butanol extract (40 g) was subjected to CC over Diaion HP-20; the column was successively eluted with H_2O, H_2O–MeOH (4:1→1:4), and finally with MeOH (500 mL each). Each fraction of 50 mL was collected and concentrated under reduced pressure. The methanol and MeOH–H_2O fractions (4:1) exhibiting identical spots of two compounds in TLC were mixed and concentrated to an oily mass (3.3 g). This oily mass was subjected to CC over silica gel and gradually eluted with $CHCl_3$–EtOAc (4:1→1:9), EtOAc, and finally with EtOAc–MeOH (19:1). The fractions from $CHCl_3$–EtOAc (1:9) and EtOAc had a similar composition on TLC with a trace of impurities. These were mixed, concentrated, and again subjected to CC over silica gel. Elution of the column with 100% EtOAc yielded compound **1** (55 mg) as yellowish fine needles.

Compound 1. Yellowish needles, mp 212°C. R_f 0.52 (EtOAc–MeOH, 9.5:0.5), $[\alpha]_D^{25}$+8° (*c* 0.32; MeOH). UV spectrum (MeOH, λ_{max}, nm): 256, 279, 350 (log ε 3.25). IR spectrum (KBr, ν, cm^{-1}): 3612 (OH), 2927 (CH), 1650 (C=O, *α*,*β* unsaturated),

1600 (C=C), 1499, 1343, 1262, 1207, 1182, 1089 (ArH). FAB-MS (+ve) (*m/z*, $I_{rel.}$, %): 493 ($[M + H]^+$, 20), 463 ($[MH – 2CH_3]^+$, 18), 331 ($[MH – 162]^+$, 100), 301 ($[MH – 162 – 2CH_3]^+$, 21), 272 (12), 181 (5), 178 (15), 154 (25). These observations along with other ^1H and ^{13}C NMR spectroscopic data (Table 1) led us to establish that **1** has a flavone-7-*O*-β-D-glucopyranoside structure [6].

Hydrolysis of Compound 1. Compound **1** (5 mg) was refluxed in 5 mL of 2 M HCl (MeOH–H_2O, 1:1) for 1 h in a water bath. The mixture was evaporated to dryness under vacuum, redissolved in water, neutralized with Ag_2CO_3, and centrifuged. The supernatant was dried under vacuum, again dissolved in H_2O, and subjected to TLC using $CHCl_3$–MeOH–H_2O–AcOH (16:9:2:2) as developing solvent. The developed TLC plate was sprayed with α-naphthol–H_2SO_4 and heated for 3 min in a hot air oven. Glucose (R_f 0.52) was identified by co-TLC with an authentic sugar.

Antioxidant Activity. DPPH radical scavenging activity was measured according to the method of Cotelle et al. with some modifications. In brief, 750 μL reaction mixture containing 50 μL of DPPH (100 μM in methanol) and 700 μL of **1** (at various concentrations; 10–200 μg/mL) was incubated at 37°C for 30 min, and absorbance was measured at 517 nm using a UV-VIS spectrophotometer. DPPH radical scavenging percentage was calculated by comparing the results of the test with those of the control (not treated with **1**) using the following formula

$$\% \text{ Inhibition} = (\text{OD of control} - \text{OD of test}) \times 100/\text{OD of control}.$$

BHT was used as positive control in the experiment.

Furin Assay. The fluorescence-based furin inhibition was measured according to the method described in the literature [14]. In brief, 50 μL reaction mixture containing 10 μL of Boc-RVRRMCA (Boc = tertiary butyloxycarbonyl, MCA = 4-methylcoumaryl-7-amide) from one of the three sets of different concentrations (60, 40, and 20 μM in DMSO), 30 μL of Tris-Mes buffer (pH 7.4) consisting of 25 mM Mes, 25 mM Tris, and 2.5 mM $CaCl_2$, 5 μL of furin, and 5 μL of **1** (at various concentrations; 10–200 μg/mL) was incubated at 37°C for 30 min, and fluorescence was measured with a UV-VIS spectrophotometer at fixed wavelengths of 370 nm and 460 nm for excitation and emission, respectively. All assays were performed at 37°C in 96 flat bottom black polystyrene microwell plates using the stop time method.

ACKNOWLEDGMENT

The work was supported by a grant (EMR-II/2389, 07.06.2010) from CSIR, New Delhi, Government of India. The authors are thankful to Dr. N. K. Chakraborty, Retired Botanist, M. B. B. College, Agartala, Tripura, and Prof. B. K. Datta, Tripura University, India for authentication of the plant material and IICB, Kolkata for spectral facilities.

REFERENCES

1. *Flora of China*, **24**, 2000, p. 379–380.
2. D. B Deb, *The Flora of Tripura State*, **2**, Today & Tomorrow′s Printers and Publishers, New Delhi, India, 1983.
3. S. Bhowmik, B. K. Datta, and N. C. Mandal, *Acta Bio. Ind.*, **2**, 363 (2013).
4. K. R. Markham, *Techniques of Flavonoid Identification*, Academic Press Inc, London, New York, 1982, p. 10003.
5. K. R. Markham, B. Ternaj, R. Stanley, H. Geiger, and T. J. Marby, *Tetrahedron*, **34**, 1394 (1978).
6. J. B. Harborne and T. J. Mabry, *The Flavonoids: Advances in Research,* Chapman and Hall, New York, 1982, p. 37.
7. H. Yoon, S. Eom, J. Hyun, G. Jo, D. Hwang, S. Lee, Y. Yong, J. C. Park, Y. H. Lee, and Y. Lim, *Bull. Kor. Chem. Soc.,* **32**, 2101 (2011).
8. Y. Park, B-Ho. Moon, E. Lee, S. Hong, S. Lee, and Y. Lim, *Bull. Kor. Chem. Soc.,* **29**, 81 (2008).
9. U. Rawat, S. Semwal, D. K. Semwal, R. Badoni, and A. Bamola, *Molbank,* **M622**, 1 (2009).
10. N. G. Seidah, *Nat. Rev. Drug Discov.*, 5 (2012).
11. N. G. Seidah, *Ann. N.Y. Acad. Sci*., **1220**, 149 (2011).
12. S. Majumdar, B. C. Mohanta, D. R. Chowdhury, R. Banik, B. Dinda, and A. Basak, *Curr. Med. Chem.*, **17**, 2049 (2010).
13. Z. A. Khushbaktova, S. M. Yusupova, M. V. Zamaraeva, E. T. Tadzhibaeva, V. N. Syrov, E. Kh. Batirov, and M. P. Yuldashev, *Chem. Nat. Compd.*, **32**, 338 (1996).
14. A. Basak, A. Chen, S. Majumdar, and H. P. Smith, *Methods Mol. Biol*., **768**, 127 (2011).