Phytochemistry Letters 12 (2015) 129–132

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Two new diterpenoids from the roots of *Pygmacopremna herbacea*

Kovela Satish [a], Galla Srihari [a], Gangarajula Sudhakar [a], Kura Narsimha [b], Tadikamalla Prabhakar Rao [b], Madugula Marthanda Murthy [a,*]

[a] Crop Protection Chemicals Division (Organic Chemistry-II), CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India
[b] Center for Nuclear Magnetic Resonance, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India

ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form 21 February 2015
Accepted 6 March 2015
Available online 18 March 2015

Keywords:
Pygmacopremna herbacea
Verbenaceae
Diterpenoids
Neobharangi-δ-lactone
Bharangi quinone
Bharangin
Neobharangin

ABSTRACT

Two new diterpenoids, neobharangi-δ-lactone (**1**) and bharangi quinone (**2**) along with two known compounds neobharangin (**3**) and bharangin (**4**) were isolated from the ethyl acetate extract of root nodules of *Pygmacopremna herbacea*. The structures of the new compounds were established by 1D and 2D NMR spectroscopic data.

1. Introduction

The Verbenaceae family is a rich source of terpenoids and quinonemethides. *Pygmacopremna herbacea* syn. *Premna herbacea* belonging to the family of Verbenaceae is a small herb or sometimes an under shrub, arising from a perennial rootstock distributed through the subtropical Himalayas, Assam, West Bengal, Bihar, Orissa and Deccan peninsula (Krishnamurthi, 1969). It is known as Gantubharangi in Telugu, Bharangi in Hindi. *P. herbacea* roots are used in the ayurvedic system and also a folk medicine against inflammatory and malaria in the Yunnan province of China.

The roots of this plant are used in the preparations of ayurvedic medicines either alone or as an ingredient for the treatment of bronchitis, asthma, blood pressure, tumors, inflammation, hiccough, epilepsy, helimenthiasis, etc. (Nayar et al., 1976). Fresh root stocks and roots along with ginger are given in asthma, rheumatism and dropsy. The rootstocks and root bark are used to cure toothache. The leaves are used in fevers and cough, and their poultices are applied to boils (Ambasta, 1996). Previous investigations on *P. herbacea* by our group have resulted in the isolation and characterization of bharangin (Sankaram et al., 1988a), isobharangin (Sankaram et al., 1989), bharanginin (Sankaram et al., 1988b), pygmacone (Sankaram and Marthandamurthy, 1991), bharangi-δ-lactone, bharangi-γ-lactone (Srihari et al., 2011), 3-dehydroxy isobharangin and neobharangin (Satish et al., 2011).

In order to isolate the minor active constituents, we reinvestigated this plant and obtained two new compounds (**1**) and (**2**) from the ethyl acetate extract of the root nodules. In this paper, we described the isolation and structure determination of **1** and **2** using 2D NMR techniques.

2. Results and discussion

P. herbacea root powder was extracted successively with *n*-hexane and ethyl acetate in a soxhlet apparatus. The extracts were concentrated under vacuum. The concentrated ethyl acetate extract was subjected to repeated column chromatography over silica gel resulting in the isolation of new compounds (**1**) and (**2**) along with known compounds neobharangin (**3**) (Satish et al., 2011) and bharangin (**4**) (Sankaram et al., 1988a) (Fig. 1).

Compound **1** was obtained as a brown colored semi solid, $[\alpha]_D^{25} + 17.33$ (*c* 0.15, $CHCl_3$). High resolution mass analysis of compound **1** showed the molecular ion [$(M+H)^+$], 403.2112, gave the elemental composition $C_{23}H_{31}O_6$ (calcd. 403.2115) in its

* Corresponding author. Tel.: +91 40 27193933; fax: +91 40 27193382.
E-mail address: marthanda52@yahoo.com (M. Marthanda Murthy).

http://dx.doi.org/10.1016/j.phytol.2015.03.010
1874-3900/

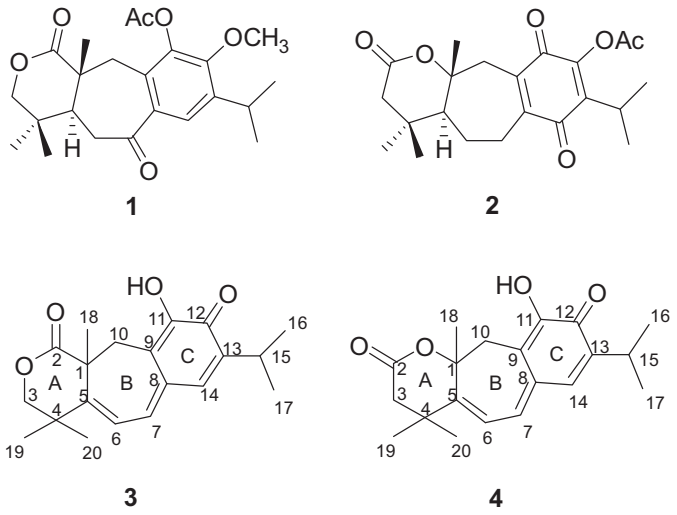

Fig. 1. Structures of isolated compounds from *P. herbacea*.

spectrum. The UV spectrum showed absorption maxima at 267 nm (log ε 3.60). The IR spectrum of **1** showed absorption bands at ν_{max}: 1737, 1687 and 1771 cm^{-1} indicating the presence of δ-lactone, saturated carbonyl and carbonyl of acetate groups, respectively. The comparison of ^{1}H NMR spectrum of **1** and **3** (Satish et al., 2011) showed a notable difference in AB doublets present in the B ring at δ_H 6.44 (1H, d, J = 6.8 Hz) of H-6 and δ_H 6.61 (1H, d, J = 6.8 Hz) of H-7 in the structure of **3** were replaced by methylene groups at C-6, δ_H 2.74 (1H, dd, J = 12.6, 17.7 Hz) and δ_H 2.67 (1H, dd, J = 3.9, 17.7 Hz) and saturated carbonyl group at C-7 in the structure of compound **1** (Table 1). The other two AB doublets protons resonated at δ_H 5.09 (1H, d, J = 13.2 Hz) and δ_H 4.73 (1H, d, J = 13.7 Hz) of CH_2-3 and δ_H 2.80 (1H, d, J = 13.6 Hz) and δ_H 2.74 (1H, d, J = 13.6 Hz) of CH_2-10 in the structure of **3** were also observed in the structure of **1**. The ^{1}H NMR spectrum of **1** also showed one methoxyl group at δ_H 3.64 and one acetoxyl group δ_H 2.36. The remaining signals including isopropyl and three methyl groups in **1** were the same in **3**.

All the 23 carbons corresponding to its molecular formula were observed in the ^{13}C NMR spectrum (Table 2). The ^{13}C NMR spectrum showed signals for three carbonyls δ_C 196.8 (saturated carbonyl), δ_C 171.8 (carbonyl of acetate group) and δ_C 167.7 (δ-lactone), six carbon signals in the aromatic region, 13 carbon signals in the aliphatic region and one carbon signal attached to an oxygen atom. The presence of three methine, three methylene and five methyl signals were differentiated in its DEPT 135° spectrum. Among the three methine carbons, two were in aliphatic (δ_C 50.6, 27.6) region and one in aromatic (δ_C 115.2). Three methylene carbons (δ_C 87.6, 37.9 and 45.2) and the remaining five methyl carbons (δ_C 23.1, 22.9, 20.2, 25.9 and 25.3) were in the aliphatic region. Strong correlations between two methylene protons of CH_2-3 (δ_H 4.63 and 4.40), CH_2-6 (δ_H 2.74 and 2.67), CH_2-10 (δ_H 2.35 and 2.25) and methine proton CH-5 (δ_H 2.55) with CH_2-6 (δ_H 2.74 and 2.67), and methine proton H-15 (δ_H 3.04) of isopropyl group with two methyl protons of H-16 (δ_H 1.21) and H-17 (δ_H 1.20) were observed in its COSY spectrum. The NOESY spectrum showed correlations between H-3 (δ_H 4.63, 4.40) protons with H-19 (δ_H 1.14) and H-20 (δ_H 1.10); H-5 (δ_H 2.55) protons with H-19 (δ_H 1.14) and H-20 (δ_H 1.10); H_a-6 (δ_H 2.74) proton with H_b-6 (δ_H 2.67); H-6 (δ_H 2.74, 2.67) protons with H-19 (δ_H 1.14) and H-20 (δ_H 1.10); H_a-10 (δ_H 2.35) protons with H_b-10 (δ_H 2.25); H-14 (δ_H 7.34) protons with H-15 (δ_H 3.04), H-16 (δ_H 1.21) and H-17 (δ_H 1.20) and finally methine proton H-15 (δ_H 3.04) of isopropyl group correlated with both methyl groups at H-16 (δ_H 1.21), H-17 (δ_H 1.20) (Fig. 2).

Table 2
^{13}C NMR (125 MHz) data for compounds **1**, **2**, **3** and **4**.

Position C	**1** ($CDCl_3$) δ_C	**3** ($CDCl_3$) δ_C	**2** ($CDCl_3$) δ_C	**4** ($CDCl_3$) δ_C
1	46.3	46.8	82.6	83.9
2	167.7	173.2	171.2	169.5
3	87.6	70.4	43.8	42.2
4	36.6	38.9	33.6	37.3
5	50.6	159.1	57.2	159.1
6	37.9	121.0	23.1	116.9
7	196.8	136.9	24.6	137.7
8	125.8	127.8	147.5	133.6
9	142.9	123.0	138.7	113.7
10	45.2	45.7	39.3	38.5
11	148.2	146.0	179.5	147.2
12	136.4	178.4	148.3	178.7
13	142.5	142.2	140.5	141.6
14	115.2	133.4	185.5	137.6
15	27.6	26.9	25.6	26.7
16	23.1	21.7	20.4	21.3
17	22.9	21.5	20.3	21.2
18	20.2	22.2	19.1	23.4
19	25.9	33.1	32.3	29.2
20	25.3	31.1	25.7	28.0
$-O\underline{C}OCH_3$	171.8	–	168.1	–
$-OCO\underline{C}H_3$	20.4	–	20.4	–
$-O\underline{C}H_3$	51.4	–	–	–

Table 1
^{1}H NMR data for Compounds **1** (500 MHz), **2** (500 MHz), **3** (500 MHz), and **4** (500 MHz).

Position H	**1** ($CDCl_3$) δ_H (m, J in Hz)	**3** ($CDCl_3$) δ_H (m, J in Hz)	**2** ($CDCl_3$) δ_H (m, J in Hz)	**4** ($CDCl_3$) δ_H (m, J in Hz)
3	4.63 (d, 8.4)	5.09 (d, 13.2)	2.55 (d, 16.4)	2.59 (d, 16.0)
	4.40 (d, 8.4)	4.73 (d, 13.7)	2.46 (d, 16.4)	2.45 (d, 16.0)
5	2.55 (dd, 12.6, 3.9)	–	1.78 (dd, 12.2, 2.6)	–
6	2.67 (dd, 17.7, 3.9)	6.44 (d, 6.8)	1.99 (m)	6.14 (d, 9.0)
	2.74 (dd, 12.6, 17.7)		1.25 (m)	
7	–	6.61 (d, 6.8)	3.45 (dd, 14.8, 6.4)	6.50 (d, 9.0)
			2.12 (t, 12.7)	
10	2.35 (d, 13.8)	2.80 (d, 13.6)	3.54 (d, 13.7)	3.67 (d, 15.0)
	2.25 (d, 13.8)	2.74 (d, 13.6)	2.45 (d, 13.7)	2.88 (d, 15.0)
14	7.34 (s)	6.88 (s)	–	6.80 (s)
15	3.04 (sept., 6.8)	3.12 (sept., 6.9)	3.17 (sept., 7.1)	3.08 (sept., 6.0)
16	1.21 (d, 6.7)	1.19 (d, 5.5)	1.23 (d, 7.1)	1.16 (d, 6.0)
17	1.20 (d, 6.7)	1.18 (d, 6.2)	1.21 (d, 7.1)	1.13 (d, 6.0)
18	1.45 (s)	1.71 (s)	1.26 (s)	1.40 (s)
19	1.14 (s)	1.48 (s)	1.12 (s)	1.33 (s)
20	1.10 (s)	1.43 (s)	0.99 (s)	1.27 (s)
–OAc	2.36 (s)	–	2.35 (s)	–
–OMe	3.64 (s)	–	–	–