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New 19α-hydroxyursane-type triterpenes from the leaves of *Meyna spinosa* (= *Vangueria spinosa*), Rubiaceae

Prasenjit Rudrapaul [a], Niranjan Das [b], Utpal Chandra De [a], Biswanath Dinda [a,c,*]

[a] Department of Chemistry, Tripura University, Suryamaninagar 799022, Tripura, India
[b] Department of Chemistry, Netaji Subhash Mahavidyalaya, Udaipur 799120, South Tripura, India
[c] Department of Chemistry, National Institute of Technology, Agartala, Jirania 799055, Tripura, India

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ABSTRACT

Two new 19α-hydroxyursane-type triterpenes, 2α,3α,19α,24,28-pentahydroxyurs-12-ene (**1**) and meyanthic acid, 3β-acetoxy-2β,19α,23-trihydroxyurs-12-en-28-oic acid (**2**) along with one new aliphatic ester, myricyl pentadecanoate (**3**) and five known compounds, 19α-hydroxyasiatic acid (**4**), oleanolic acid (**5**), myricyl alcohol (**6**), β-sitosterol (**7**) and its glycoside (**8**) were isolated from the methanolic leaf extract of *Meyna spinosa* Roxb. ex Link (= *Vangueria spinosa* Roxb., Rubiaceae). The structures of the new compounds were elucidated on the basis of extensive spectroscopic (including 2D NMR) analysis and comparison with literature. Except oleanolic acid, isolation of known compounds was reported for the first time from this plant.

1. Introduction

Meyna spinosa Roxb. ex Link (= *Vangueria spinosa* Roxb., Rubiaceae), a medium sized thorny bushy shrub, is distributed in East and South India, and in neighboring countries Bangladesh, Nepal, Java, Thailand, Vietnam and Myanmar (Deb, 1983). In Assam (India), the plant is known as "Kutkura" and in Bangladesh as "Moyna". Ripe fruits have slightly sweet in taste and are eaten raw. In traditional medicine, the mature fruits are used for treatment of gastritis, cracked heels, piles, biliary and hepatic congestions (Buragohain, 2008; Yusuf et al., 2009). Leaves are used for the treatment of bone-fracture, skin irritation, abortion, diphtheria and renal diseases (Pullaiah, 2002). Previous studies on the plant reported the isolation of (-) epicatechin-3-*O*-β-D-glucopyranoside from leaves (Chatterjee et al., 2011), oleanolic acid from fruits (Buragohain, 2008) and saponin, 3β-*O*-[α-L-rhamnopyranosyl-(1→2) β-D-xylopyranosyl]-oleanolic acid from leaves (Gogoi and Sarma, 1997). We chemically investigated the leaves of the plant and isolated two new 19α-hydroxyursane-type triterpenes **1** and **2** as well as a new aliphatic ester **3** and five known compounds, **4**–**8** from the methanolic extract. We report in this paper, the isolation and structure elucidation of these compounds.

2. Results and discussion

The powdered leaves of *M. spinosa* were successively extracted with methanol at room temperature and the methanolic extract was fractionated into petroleum ether, chloroform, ethyl acetate and *n*-butanol fractions. Compounds **3**, **6**, **7** were isolated from the petroleum ether extract and compounds **1**, **2**, **4**, **5** and **8** (Fig. 1) from chloroform extract after extensive silica gel column chromatography. Compounds **4** and **5** were also obtained from ethyl acetate extract.

Known compounds **4**, **5**, **6**, **7** and **8** were identified as 19α-hydroxyasiatic acid (2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid) (Gao et al., 1985), oleanolic acid (Mahato and Kundu, 1994; Maillard et al., 1992), myricyl alcohol (Dinda et al., 1999), β-sitosterol (Zhang et al., 2006) and 3-*O*-β-D-glucopyranosyl-β-sitosterol (Paulo et al., 2000), respectively, by comparison of their ^{1}H and ^{13}C-NMR spectra and mass spectral fragmentation patterns with the reported data.

Compound **1** (Fig. 1) was obtained as colorless amorphous powder. Its molecular formula was determined as $C_{30}H_{50}O_5$ from the HR-FAB-MS at *m/z* 491.3733 $[M+H]^+$ (Calcd. for $C_{30}H_{51}O_5$, 491.3737) and ^{13}C-NMR spectral data. The IR spectrum showed characteristic absorptions for hydroxyl (3436 (br) cm^{-1}) and olefinic (1636 cm^{-1}) functions. The ^{1}H-NMR spectrum (Table 1) showed one proton singlet at δ_H 2.68 along with five tertiary methyl singlets at δ_H 1.05, 1.18, 1.34, 1.45 and 1.69, a secondary methyl at δ_H 0.93 (d, J = 6.6 Hz, H_3-30) and an olefinic proton signal

* Corresponding author at: Department of Chemistry, Tripura University, Suryamaninagar 799022, Tripura, India. Tel.: +91 9436502137.
E-mail address: dindabtu@gmail.com (B. Dinda).

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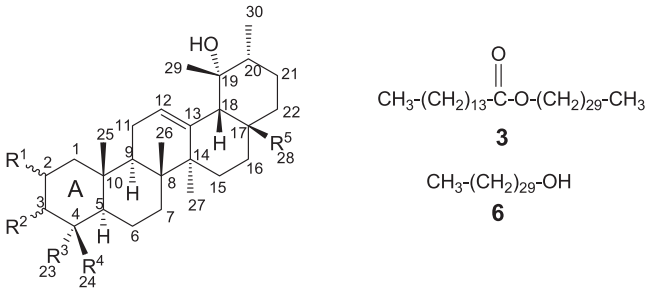

	R^1	R^2	R^3	R^4	R^5
1	α-OH	α-OH	CH_3	CH_2OH	CH_2OH
2	β-OH	β-OAc	CH_2OH	CH_3	COOH
4	α-OH	β-OH	CH_2OH	CH_3	COOH

Fig. 1. Structures of compounds **1–4** and **6.**

at δ 5.50 (br s), characteristic of 19α-hydroxyursane type-triterpene (Jung et al., 2004). The ^{1}H-NMR spectrum also exhibited signals for two carbinol methines at δ_H 4.21 (1H, ddd, J = 10.0, 5.4, 3.0 Hz) and 3.83 (1H, d, J = 3.0 Hz), assignable to H-2β and H-3β, respectively (Kojima and Ogura, 1989). The coupling constants $^3J_{H\text{-}1/H\text{-}2\ (trans)}$ = 10.0 Hz, $^3J_{H\text{-}1/H\text{-}2\ (cis)}$ = 5.4 Hz and $^3J_{H\text{-}2/H\text{-}3\ (cis)}$ = 3.0 Hz were consistent for 2α,3α-dihydroxyl substituents in ring A (Kojima and Ogura, 1989). Furthermore, two proton signals at δ_H 3.72 (1H, d, J = 10.8 Hz) and 4.17 (1H, d, J = 10.8 Hz) in the ^{1}H-NMR spectrum suggested the presence of a hydroxymethyl group at 23 or 24 position. The ^{13}C-NMR spectrum (Table 1) showed 30 carbons including six methyl carbons (δ 16.3, 17.1, 17.8, 24.4, 25.0, 27.4) and two sp^2 carbons of a trisubstituted olefin at δ_C 127.2 and 138.4, one tertiary carbon at δ 73.0, two hydroxymethyl carbons at δ 65.3 and 68.1 and two methine carbons bearing a hydroxyl group at δ 68.0 and 73.8 suggesting its 19α-hydroxyurs-12-ene-type triterpenoid structure with two secondary hydroxyl groups and two hydroxymethyl groups for the compound (Cheng and Cao, 1992). The presence of carbon signals in **1** at δ_C 24.4 (CH_3) and 65.3 (CH_2) suggested the presence of hydroxyl group at C-24 position (Kojima and Ogura, 1989; Zhang and Yang, 1994). The additional two proton signals at δ_H 3.30 (1H, d, J = 10.2 Hz) and 3.62 (1H, d, J = 10.2 Hz) in its ^{1}H-NMR spectrum, and a methylene carbon signal at δ_c 68.1 suggested the presence of a hydroxymethyl group. Its location at C-28 position was assigned by HMBC correlation of H-18 with δ_C at 68.1. The HMBC correlation of H-3 with δ_C at 65.3 also supported the location of another hydroxymethyl group at 24-position. The ^{13}C-NMR data were very similar to that of 2α,3α,19α,24-tetrahydroxyurs-12-en-28-oic-28-β-D-glucopyranosylester except at C-28 (Jung et al., 2004). The NOESY correlations between H-2 and H-25; H-3 and H-24 and between H-18 and H-28 of **1** also supported the assignments of 2α,3α,24,28-hydroxyl groups in the compound. Furthermore, NOESY relations between H-18 and H-19 indicated the α-orientation of C-19 hydroxyl group. Selected NOESY correlations in **1** are shown in Fig. 2. Thus, compound **1** was determined to be 2α,3α,19α,24,28-pentahydroxyurs-12-ene (**1**).

Table 1
NMR spectroscopic data[a] of compounds **1** and **2** (in C_5D_5N) (δ, ppm; J, Hz).

Position	**1**		2	
	δ_C	δ_H	δ_C	δ_H
1/CH_2	42.4	3.11 (m), 1.25 (m)	42.9	2.31 (dd, 12.6, 4.2) 1.25 (dd, 12.6, 2.4)
2/CH	68.0	4.21 (ddd, 10.0, 5.4, 3.0)	69.2	4.27 (ddd, 4.2, 4.0, 2.4)
3/CH	73.8	3.83 (d, 3.0)	78.6	5.06 (d, 2.4)
4/C	42.8	–	42.5	–
5/CH	48.9	1.60 (m)	48.3	2.05 (m)
6/CH_2	19.0		19.0	
7/CH_2	33.5		33.5	
8/C	40.2	–	40.8	–
9/CH	48.3	1.74 (m)	48.6	1.77 (m)
10/C	38.8		38.8	
11/CH_2	24.3		24.5	
12/CH	127.2	5.50 (br s)	128.3	5.60 (t-like)
13/C	138.4	–	140.3	–
14/C	42.6	–	42.5	–
15/CH_2	27.9		29.6	
16/CH_2	24.2	–	26.7	
17/C	48.6	–	48.2	
18/CH	54.8	2.68 (s)	54.9	3.06 (s)
19/C	73.0	–	73.0	–
20/CH	43.2		42.7	
21/CH_2	30.3		27.3	
22/CH_2	37.5		38.7	1.87 (br d, 11.4) 1.74(dd, 11.4, 4.2)
23/CH_3/CH_2	24.4	1.69 (s)	66.6	3.74 (d, 10.2) 4.22 (d, 10.2)
24/CH_2/CH_3	65.3	4.17 (d, 10.8) 3.72 (d, 10.8)	14.7	1.43 (s)
25/CH_3	17.8	1.05 (s)	17.7	1.11 (s)
26/CH_3	17.1	1.18 (s)	17.6	1.09 (s)
27/CH_3	25.0	1.45 (s)	25.0	1.15 (s)
28/CH_2/C	68.1	3.62 (d, 10.2) 3.30 (d, 10.2)	181.0	–
29/CH_3	27.4	1.34 (s)	27.4	1.67 (s)
30/CH_3	16.3	0.93 (d, 6.6)	17.1	1.12 (d, 6.0)
19/OH		5.82 (br s)		6.29 (s)
3/OAc			21.7, 170.2	2.05 (s)

[a] Assignment based on HSQC, HMBC and DEPT experiments; 600 MHz for ^{1}H- and 150 MHz for ^{13}C-NMR data.