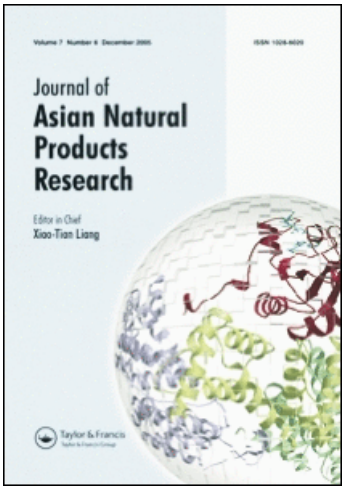

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

A new pregnane glycoside from *Marsdenia roylei* as potential antioxidant and antidyslipidemic agents

Arun Sethi[a]; Sudha Paswan[a]; Sanjay Srivastava[b]; Naveen Kumar Khare[a]; Akriti Bhatia[a]; Alok Kumar[c]; Geetika Bhatia[d]; Mohammad Mubin Khan[d]; Ashok Kumar Khanna[d]; Jitendra Kumar Saxena[d]
[a] Department of Chemistry, University of Lucknow, Lucknow, India [b] Department of Applied Sciences, Institute of Engineering and Technology, Lucknow, India [c] Department of Chemistry, Northern India Engineering College, Lucknow, India [d] Division of Biochemistry, Central Drug Research Institute, Lucknow, India

To cite this Article Sethi, Arun , Paswan, Sudha , Srivastava, Sanjay , Khare, Naveen Kumar , Bhatia, Akriti , Kumar, Alok , Bhatia, Geetika , Khan, Mohammad Mubin , Khanna, Ashok Kumar and Saxena, Jitendra Kumar(2008) 'A new pregnane glycoside from *Marsdenia roylei* as potential antioxidant and antidyslipidemic agents', Journal of Asian Natural Products Research, 10: 11, 1023 — 1028
To link to this Article: DOI: 10.1080/10286020802280190
URL: http://dx.doi.org/10.1080/10286020802280190

Journal of Asian Natural Products Research
Vol. 10, No. 11, November 2008, 1023–1028

A new pregnane glycoside from *Marsdenia roylei* as potential antioxidant and antidyslipidemic agents

Arun Sethi[a]*, Sudha Paswan[a], Sanjay Srivastava[b], Naveen Kumar Khare[a], Akriti Bhatia[a], Alok Kumar[c], Geetika Bhatia[d], Mohammad Mubin Khan[d], Ashok Kumar Khanna[d] and Jitendra Kumar Saxena[d]

[a]*Department of Chemistry, University of Lucknow, Lucknow, India;* [b]*Department of Applied Sciences, Institute of Engineering and Technology, Lucknow, India;* [c]*Department of Chemistry, Northern India Engineering College, Lucknow, India;* [d]*Division of Biochemistry, Central Drug Research Institute, Lucknow, India*

(*Received 11 December 2007; final version received 8 May 2008*)

A new pregnane glycoside roylenine (**1**) was isolated from the $CHCl_3$–EtOH (4:1) extract of *Marsdenia roylei*. Its structure was established on the basis of spectroscopic studies. The glycoside (**1**) and its acetylated derivative (**6**) were evaluated for their antioxidant and antidyslipidemic activities.

Keywords: asclepiadaceae; *Marsdenia roylei*; pregnane glycoside; roylenine; antioxidant; antidyslipidemic

1. Introduction

Asclepiadaceae family plants are a rich source of cardiac [1] and pregnane glycosides [2]. These pregnanes and their glycosides, which are known to possess antitumor and anticancer activities [2,3], have also shown antiproliferative activity [4,5] on J774, A1, HEK-293 and WEHI-164 cell lines. *Marsdenia roylei* (family Asclepiadaceae) is widely used in ayurveda and unani medicine. The juice isolated from its stems is used in the treatment of gastric trouble and peptic ulcers [6].

Our continuing studies [7–9] on pregnane glycosides from *M. roylei* led to the isolation of roylenine (**1**); herein its structural elucidation is reported.

2. Results and discussion

Roylenine (**1**), m.p. 119–123°C, $[\alpha]_D$ +10, $C_{37}H_{58}O_{12}$, FAB-MS *m/z* 756 [M^+ + Na + K], responded positively to Liebermann–Burchardt [10], xanthydrol [10], Keller–Killiani [10], and Feigl tests [2], indicating it to be a steroidal glycoside of 2,6-dideoxy and normal hexose(s). The presence of two anomeric protons and carbons at δ 5.45 and 4.67 and δ 102.1 and 97.4 in its ^{1}H and ^{13}C NMR spectra, respectively, suggested **1** to be a diglycoside. The presence of two singlets of three protons each at δ 2.17 and 2.08 in the ^{1}H NMR spectrum, carbon signals at δ 171.2, 170.1, and 22.7 and 22.0 in the ^{13}C NMR spectrum suggested the presence of two acetyl functions in **1**. The aglycone and monosaccharides were identified using the Mannich and Siewert [2] method of hydrolysis, which was completed in 7 days showing three spots on TLC, of which two were identified as calogenin (**3**) and L-fucose (**4**) by comparison of their TLC, m.p., and [α] values with published data [7]. The third less polar spot, though not identified at this stage, was

*Corresponding author. Email: alkaarunsethi@rediffmail.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802280190
http://www.informaworld.com

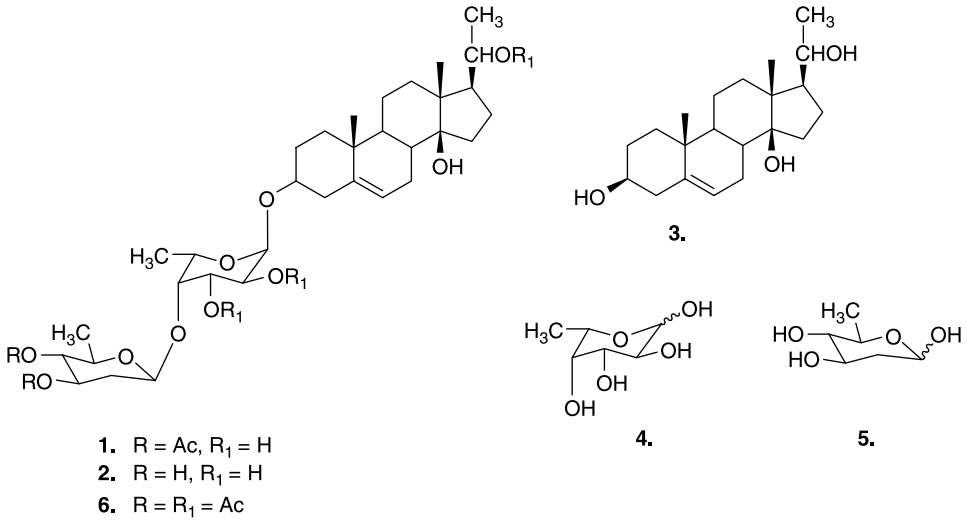

Figure 1. Structures of compounds **1-6**.

presumably the esterified terminal monosaccharide. To identify this component, **1** was subjected to mild alkaline hydrolysis, which gave **2**, $C_{33}H_{54}O_{10}$. The difference of $2 \times COCH_3$ between the molecular formula of **1** and **2** confirmed **1** to be di-*O*-acetyl derivative of **2**. Mild acid hydrolysis of **2** afforded three chromatographically pure products that were identified as calogenin (**3**) [11,12], L-fucose (**4**) and D-canarose (**5**). These results confirmed that D-canarose carried the two acetyl functions at the only available hydroxyl groups at C-3 and C-4 and is the terminal monosaccharide, linked through L-fucose to calogenin. The monosaccharides were confirmed by preparing their known derivatives [12,7]. The presence of acetyl functions at the C-3 and C-4 were confirmed by the ^{1}H NMR spectrum of **1**, which showed a downfield shifted one proton triplet at δ 5.03 having a large coupling of 9.0 Hz, typical of a triaxial (H-3, H-4, H-5) conformation, and being due to the methine proton at C-4. Similarly, the downfield shifted one proton multiplet at δ 4.16–4.25 was due to C-3 methine proton.

The ^{1}H NMR spectrum of **1** showed a one-proton doublet at δ 5.45 ($J = 3.0$ Hz) and one double doublet at δ 4.67 (1H, $J = 8.0$ and 2.0 Hz) due to the anomeric protons of L-fucose and D-canarose. The small coupling constant of the anomeric doublet due to L-fucose and the large coupling constant of the double doublet due to D-canarose showed that L-fucose and D-canarose were linked through α-glycosidic linkage in $^1C_4$ and β-glycosidic linkage in $^4C_1$ conformations, respectively. For convenience, esterified D-canarose and L-fucose are designated S2 and S1, respectively.

The ^{13}C NMR spectrum (Table 3) of **1** showed a peak at δ 79.7 due to C-3 of the calogenin. The downfield glycosidation shift of this peak showed that the sugar chain was linked to the C-3 hydroxyl of the genin. Similarly, the signal due to the C-4 of the L-fucose was also shifted downfield at δ 84.3 clearly establishing that C-1 of esterified D-canarose moiety is glycosidically linked to the C-4 hydroxyl function of L-fucose. The point of attachment was further confirmed by preparing the acetyl derivative **6** of roylenine. The ^{1}H NMR spectrum of **6** showed five acetyl group signals at δ 2.20, 2.17, 2.15, 2.08, and 2.06. It also showed the downfield shifting of C-20 methine proton of the aglycone from δ 3.74–3.78 to δ 4.15–4.10, while C-2 and C-3 protons of L-fucose were shifted downfield from δ 3.17 (dd, $J = 9.0$ and 3.0 Hz) to δ 3.66 (dd, $J = 9.0$ and 3.0 Hz) and δ 3.64 (dd, $J = 9.0$ and 2.0 Hz) to δ 4.28 (dd, $J = 8.0$ and 2.0 Hz). These results clearly established that C-1 of esterified

D-canarose is linked to the C-4 hydroxyl of L-fucose, which in turn was linked to the C-3 hydroxyl function of calogenin.

The FAB-MS of **1** recorded the alkali metal cationized molecular ion peak at *m*/*z* 756. An important mass ion fragment that arose by rearrangement involving migration of the acetyl group of S2 at C-3 to C-1 of the same sugar, after the radical ion fission of C1—C2 bond [2], resulting in the cleavage of the terminal sugar appeared at *m*/*z* 553. This fragment further confirmed that the esterified D-canarose is the terminal sugar. The mass ion peak at *m*/*z* 327 was due to the mass ion fragment of the monoglycoside (obtained by the loss of S2) followed by subsequent losses of two angular methyls, two water molecules, one CH_3CHO, and the C-17 side chain. Thus, confirming the attachment of sugar moiety to the C-3 hydroxyl of the aglycone. Based on the foregoing evidence, the structure of **1** was established as calogenin-3-*O*-(3,4-di-*O*-acetyl)-β-D-canaropyranosyl-(1 → 4)-α-L-fucopyranoside.

3. Biological activity

The present study was undertaken to evaluate the antidyslipidemic and antioxidant activities of pregnane glycoside. The procedure adopted was the same as reported earlier [13].

3.1 Lipid-lowering activity

Rats were divided into five groups: control, triton induced, triton plus **1**, **6**, and gemfibrozil-treated (100 mg/kg) groups containing two rats in each group. Pregnane glycosides and gemfibrozil were macerated with gum acacia, suspended in water, and fed simultaneously with triton at a dose of 100 mg/kg p.o. to the animals.

3.2 Antioxidant activity

Superoxide anions (O^{2-}) were generated enzymatically by xanthine (160 mM), xanthine oxidase (0.04 units), and nitroblue tetrazolium (320 μM) in the absence or presence of compounds **1** and **6** (200 μg/ml)

Table 1. Lipid-lowering activity of pregnane glycosides in triton-treated hyperlipidemic rats.

Treatment	Total cholesterol (Tc)	Phospholipid (Pl)	Triglyceride (Tg)
Control	84.62 ± 6.27	75.34 ± 6.77	80.22 ± 5.62
Triton treated	332.22 ± 20.88 (+3.92F)***	270.70 ± 18.77 (+3.59F)***	332.22 ± 20.88 (+3.74F)***
Triton + **1**	277.65 ± 22.16 (− 16)*	240.33 ± 19.73 (− 11)*	246.96 ± 15.06 (− 17)*
Triton + **6**	240.39 ± 20.28 (− 27)*	215.63 ± 17.08 (− 20)*	231.99 ± 23.50 (− 22)*
Triton + gemfibrozil (100 mg/kg) standard drug	240.39 ± 20.28 (− 34)***	240.39 ± 20.28 (− 35)***	240.39 ± 20.28 (− 37)***

Unit: mg/dl. Each value is mean ± SD of two rats. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. NS, non-significant.

Table 2. Antioxidant activity of pregnane glycosides *in vitro*.

Treatment	Concentration	Formation of superoxide anions[a]	Formation of hydroxyl radicals[b]
1	None	42.50 ± 3.20	6.10 ± 0.07
	200	40.97 ± 2.81 (−4)NS	6.14 ± 0.03 (−0.78)NS
6	None	18.75 ± 1.12	5.40 ± 0.04
	200	14.19 ± 1.00 (−25)***	4.36 ± 0.02 (−19)**
Alloperinol (20 μg/ml)	None	26.55 ± 0.57	
	20	2.60 ± 0.41 (−90)***	
Mannitol (100 μg/ml)	None		32.62 ± 1.31
	100		17.12 ± 2.68 (−48)***

Units: [a]nmol formazone formed/minute; [b]*n* mol MDA/h. Each value is the mean ± SD of four separate observations. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. NS, non-significant when compared with the systems without drug treatment.

in 100 mM phosphate buffer (pH 8.2). In another set of experiments, the effect of these compounds (200 μg/ml) on generation of hydroxyl radical (OH·) was also studied by non-enzymic reactants.

3.3 Effect of pregnane glycosides on hyperlipidemia

Administration of triton WR-1339 in rats induced marked hyperlipidemia as evidenced by the increase in the plasma level of Tc (3.92-fold), Pl (3.59-fold), and Tg (3.74-fold) when compared with control (Table 1). Treatment of hyperlipidemic rats with pregnane glycosides at the dose of 100 mg/kg p.o. reversed the plasma levels of lipid with varying extents (Table 1). These data compared with standard drug gemfibrozil at the dose of 100 mg/kg showed decrease in the plasma levels of Tc, Pl, and Tg by 34, 35, and 37%, respectively. The order of

Table 3. ^{13}C NMR spectral data of **1** in $CDCl_3$.

Aglycone *Position*		L-Fuc		D-Can	
1	36.5, t	1′	97.4, d	1″	102.1, d
2	29.3, t	2′	67.7, d	2″	40.2, t
3	79.7, d	3′	69.7, d	3″	78.4, d
4	39.2, t	4′	84.3, d	4″	82.2, d
5	139.1, s	5′	66.8, d	5″	70.6, d
6	122.5, d	6′	20.1, q	6″	18.4, q
7	29.3, t			*C*O	170.1, s
8	32.5, d			*C*O	171.2, s
9	53.7, d			CO*C*H_3	22.0, q
10	38.0, s			CO*C*H_3	22.7, q
11	22.9, t				
12	37.9, t				
13	52.2, s				
14	85.1, d				
15	31.9, t				
16	28.4, t				
17	53.4, d				
18	13.6, q				
19	17.9, q				
20	67.2, d				
21	19.8, q				

Multiplicity was determined by DEPT experiments (s, quaternary; d, methine; t, methylene; q, methyl).

lipid-lowering activity by these pregnane glycosides in the above model was **6** > **1** (Table 1).

3.4 Effect of 1 and 6 on oxygen free radical generation in vitro

The scavenging potential of pregnane glycosides at 200 μg/ml against formation of O^{2-} and OH· in non-enzymic system was also studied (Table 2). Compound **6** showed antioxidant activity in the above tests (Table 2).

4. Experimental

4.1 General experimental procedures

The melting points are recorded on an electrically heated melting point apparatus and are uncorrected. Optical rotations were recorded on ORIBA, SEPA-300 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DRX-300 MHz spectrometer using TMS as an internal reference. FAB-MS was recorded on JEOL SX 102/DA 6000 mass spectrometer. Chemical analysis was carried out on Carlo Erba 1108 instrument. Solvents used were of laboratory grade, purified, and dried according to standard procedures. Column chromatography was performed with silica gel (60–120 mesh). Paper chromatography was conducted on Whatman No. 1 paper.

4.2 Plant material, extraction, and isolation

Stems of mature *M. roylei* were collected from Dehradun forest, India. The method of extraction is the same as reported earlier [7]. Fractionation of crude extract of *M. roylei* yielded $CHCl_3$–EtOH (4:1) extract (1.03 g). Repeated column chromatography of the extract using different polarities of $CHCl_3$–MeOH as eluent afforded **1** (87 mg).

Table 4. ^{1}H NMR spectral data of compounds **1**, **2**, and **6**.

Position	1	2	6
3	4.25–4.16, m	4.18–4.12, m	4.26–4.17, m
6	5.35, m	5.38, m	5.36, m
18	1.0, s	1.08, s	0.99, s
19	0.87, s	0.82, s	0.85, s
20	3.78–3.74, m	3.76–3.72, m	4.15–4.10, m
21	1.15, d, 6.0	1.14, d, 6.2	1.16, d, 6.2
1′	5.45, d, 3.0 Hz	5.47, d, 3.0	5.52, d, 3.0
2′	3.17, dd, 9.0, 3.0	3.18, dd, 9.0, 3.0	3.66, dd, 9.0, 3.0
3′	3.64, dd, 8.0, 2.0	3.68, dd, 8.0, 2.0	4.28, dd, 8.0, 2.0
4′	3.81, t, 3.0	3.79, t, 3.0	3.88, t, 3.0
5′			
6′	1.25, d, 7.0	1.28, d, 6.9	1.26, d, 6.9
1″	4.67, dd, 8.0, 2.0	4.61, dd, 8.0, 2.0	4.72, dd, 8.0, 2.0
2″	Eq 2.60–2.55, m	2.62–2.58, m	1.88–1.72, m
	Ax 1.89–1.70, m	1.74–1.68, m	4.26–4.17, m
3″	4.25–4.16, m	3.36–3.32, m	5.12, t, 8.0
4″	5.03, t, 9.0	3.94, t, 9.0	
5″			
6″	1.15, d, 6.0	1.14, d, 6.2	1.16, d, 6.2
$COCH_3$	2.17, s		2.20, s
$COCH_3$	2.08, s		2.17, s
$COCH_3$			2.15, s
$COCH_3$			2.08, s
$COCH_3$			2.06, s

4.2.1 Roylenine (1)

Compound **1**, m.p. 119–123°C (MeOH). $[\alpha]_D$ +10 (*c*. 0.06, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) (Table 4). FAB-MS *m/z*: 756 [M^+ + Na + K] (10), 708 [M^+ + Na + K − $2CH_3$ − H_2O] (10), 695 [M^+ + H] (10), 694 [M^+] (15), 617 [695-CH_3COOH—H_2O] (10), 599 [617-H_2O] (15), 582 [599-H_2O] (10), 553 [695-$C_7H_{10}O_3$] (15), 538 [553-CH_3] (15), 520 [538-H_2O] (25), 502 [520-H_2O] (10), 482 [695 + H—S2] (10), 457 [502-CH_3CHOH] (10), 452 [482-$2CH_3$] (10), 434 [452-H_2O] (30), 432 [520-$2CH_3CHO$] (20), 416 [434-H_2O] (15), 379 [disaccharide$^+$ + 1] (10), 372 [416-CH_3CHO], 362 [379-OH], 344 [362-H_2O], 335 [aglycone$^+$ + 1] (10), 327 [372-CH_3CHOH] (15), 320 [335-CH_3] (10), 260 [320-CH_3—CH_3CHOH] (100), 233 [$S2^+$ + 1] (10), 216 [233-OH] (10), 173 [233-CH_3COOH] (15), 165 [$S1^+$ + 1] (25), 156 [216-CH_3COOH] (10), 147 [165-H_2O] (20), 103 [147-CH_3CHO] (15). Elemental analysis: found C, 63.96; H, 8.41%; calcd for $C_{37}H_{58}O_{12}$: C, 63.88, H, 8.37%.

4.3 Methanolic KOH hydrolysis of 1

Compound **1** (15 mg) was dissolved in 1% methanolic KOH (1.5 ml) and heated under reflux for 45 min, which, after usual workup, yielded **2** (10 mg). For ^1H NMR spectral data (300 MHz, $CDCl_3$), see Table 4. Elemental analysis: found C, 64.89; H, 8.91%; calcd for $C_{33}H_{54}O_{10}$: C, 64.78; H, 8.85%.

4.4 Mild hydrolysis of 2

To a solution of **2** (8 mg) in 80% 1,4-dioxane (0.8 ml) was added 0.05 N H_2SO_4 (0.8 ml) and the mixture was warmed at 50°C for 30 min. The usual workup [14,10] followed by column chromatography afforded calogenin (**3**, 1.1 mg, m.p. 200–203°C, $[\alpha]_D$ −50 (*c*. 0.12, MeOH)), L-fucose (**4**, 1.0 mg, $[\alpha]_D$ +72.2 (*c*. 0.10, H_2O)), and D-canarose (**5**, 1.0 mg, $[\alpha]_D$ −82 (*c*. 0.10, H_2O)).

4.5 Tri-O-acetyl roylenine (6)

Compound **1** (15 mg) on acetylation with Ac_2O (0.20 ml) in pyridine (1.5 ml) at room temperature and usual workup yielded **6** as an amorphous residue (15 mg). For ^1H NMR (300 MHz, $CDCl_3$) spectral data, see Table 4.

Acknowledgements

The authors are grateful to the RSIC division of the Central Drug Research Institute, India, for the analytical data.

References

[1] N. Kaneda, H. Chai, J.M. Pezzuto, A.D. Kinghorn, R. Farnsworth, P. Tuchinda, J. Udchachou, T. Santisuk, and V. Rentrakul, *Planta Med.* **58**, 429 (1992).
[2] D. Deepak, S. Srivastav, and A. Khare, *Prog. Chem. Nat. Prod.* **71**, 169 (1997).
[3] W. Pan, F. Chang, L. Wei, and Y. Wu, *J. Nat. Prod.* **66**, 161 (2003).
[4] M. De Leo, N. De Tommasi, R. Sanogo, G. Autore, S. Marzocco, C. Pizza, I. Movelli, and A. Braca, *Steroids* **70**, 573 (2005).
[5] A. Plaza, A. Perrone, M.L. Balestrieri, F. Felice, C. Balestrieri, A.J. Hamed, C. Pizza, and S. Piacente, *Steroids* **70**, 594 (2005).
[6] N.P. Manandhar, *Plants and People of Nepal*, (Timber Press, Oregon, 2002), p. 527, O-88192.
[7] V. Gupta, A. Kumar, D. Deepak, A. Khare, and N.K. Khare, *Phytochemistry* **64**, 1327 (2003).
[8] A. Kumar, A. Khare, and N.K. Khare, *Phytochemistry* **52**, 675 (1999).
[9] A. Kumar, A. Khare, and N.K. Khare, *Phytochemistry* **50**, 1353 (1999).
[10] K. Prakash, A. Sethi, D. Deepak, A. Khare, and M.P. Khare, *Phytochemistry* **30**, 297 (1991).
[11] S. Srivastav, D. Deepak, and A. Khare, *Tetrahedron* **50**, 789 (1994).
[12] O.P. Srivastava, A. Khare, and M.P. Khare, *J. Nat. Prod.* **45**, 211 (1982).
[13] A. Sethi, A. Maurya, V. Tewari, S. Srivastava, S. Faridi, G. Bhatia, M.M. Khan, A.K. Khanna, and J.K. Saxena, *Bioorg. Med. Chem.* **15**, 4520 (2007).
[14] A. Sethi, S.S. Srivastava, and S. Srivastava, *Ind. J. Hetro. Chem.* **16**, 191 (2006).