PERGAMON

Phytochemistry 52 (1999) 675–679

PHYTOCHEMISTRY

Trisaccharides from *Marsdenia roylei*

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Received 3 February 1999; accepted 21 April 1999

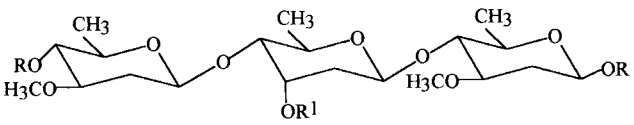
Abstract

The structures of two novel trisaccharides viz. royleose and deniose isolated from *Marsdenia roylei* were elucidated with the help of modern physico–chemical techniques and chemical transformations. They were defined as *O*-β-D-oleandropyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranose and *O*-β-D-oleandropyranosyl-(1 → 4)-*O*-β-D-digitoxopyranosyl-(1 → 4)-β-D-oleandropyranose respectively. This is the first report on the isolation of trisaccharides of rare deoxy sugars in free state from nature.

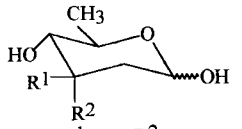
Keywords: Marsdenia roylei; Asclepiadaceae; Trisaccharides; Reducing

1. Introduction

Various species of the genus *Marsdenia* (Family: Asclepiadaceae) have been investigated chemically and a number of pregnane glycosides of deoxy hexoses (Deepak, Srivastava & Khare, 1997b) and oligosaccharides (Saner & Allgeier, 1969; Saner, Zerlentis, Stocklin & Reichstein, 1969; Singhal, Khare & Khare, 1980) have been isolated. Some of the species are known to possess medicinal properties viz. antiasthmatic, antifertility, anticancer activity and are also used against gonorrhea (Kirtikar & Basu, 1984). We now report the chemical investigation of the hitherto uninvestigated species, *Marsdenia roylei* resulting in the isolation of two novel trisaccharides **1** and **3** in their native state.



	R	R^1
1.	H	CH_3 (royleose)
2.	Ac	CH_3
3.	H	H (deniose)
4.	Ac	Ac



	R^1	R^2
5.	OCH_3	H
6.	H	OCH_3
7.	H	OH

2. Results and discussion

Royleose (**1**), $C_{21}H_{38}O_{10}$ reduces Fehlings solution and gives positive tests in the xanthydrol (Barton, Evans & Gardner, 1952) and Keller–Kiliani (Ngata, Tamm & Reichstein, 1957) reactions indicating it to be a reducing glycon containing rare 2,6-dideoxy sugar(s). The ^{13}C NMR spectrum of **1** contains three anomeric carbons at δ 101.46, 99.86 and 91,99 supplemented by three anomeric proton signals at δ 5.05, 4.81 and 4.53 in the 400 MHz ^1H NMR spectrum of **1**. The mass ion peak at m/z 473 $[M+Na]^+$ in the FAB mass spectrum of **1** also supported the nature of **1** as trisaccharide of the 2,6-dideoxy sugars. The 2,6-dideoxy nature of sugars was further confirmed by the presence of

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0031-9422/99/$ - see front matter
PII: S0031-9422(99)00253-8

characteristic methylene group signals in the region δ 2.37–2.32 (1H), 2.22–2.14 (2H) and 1.80–1.75 (1H), 1.68–1.60 (2H) for three equatorial and three axial protons respectively and three secondary methyl group doublets (J = 6 Hz) at 1.28, 1.22 and 1.20. The presence of three singlets of three protons each at 3.53, 3.45 and 3.32 for three methoxy groups in **1** showed that all the three sugars are methylated.

Mild acid hydrolysis (0.05 N H_2SO_4) (Rangaswami & Reichstein, 1949) of **1** gave D-oleandrose (**5**) (Renkonen, Schindler & Reichstein, 1959) and D-cymarose (**6**) (Krasso, Weiss & Reichstein, 1963) (TLC, PC, $[\alpha]_D$) in the ratio of 2:1. Sugars **5** and **6** were further characterized by preparing their known phenyl hydrazides D-oleandronic acid phenyl hydrazide (Renkonen et al., 1959) mp 133–136°C and D-cymaronic acid phenyl hydrazide (Krasso et al., 1963) mp 150–154°C (mp, mmp, TLC and PC).

The large coupling constants for all the three anomeric protons present as double doublets at δ 5.05, 4.81 and 4.53 (J = 9 Hz and 2 Hz) were typical of an axial configuration suggesting that all the three sugars were present in $^4C_1$ (D) conformation having β-glycosidic linkages (Allgeier, 1968).

Compound **1** on acetylation with Ac_2O gave di-*O*-acetyl derivative **2** $C_{25}H_{42}O_{12}$. The ^{1}H NMR spectrum of **2** showed the downfield shifting of one of the anomeric proton from δ 5.05 to 5.98 (dd, J = 9 Hz and 2 Hz) and of H-4″ proton from 3.15 to 4.64 (t, J = 9 Hz) showing the reducing nature of the trisaccharide and the presence of one of the D-oleandrose unit at the terminal end. The ^{1}H NMR also showed two singlets of three protons each at δ 2.10 and 2.00 for two acetoxy methyls besides showing the other signals. The sugar sequence in **1** was confirmed by the very mild acid (0.005 N H_2SO_4) hydrolysis (Rangaswami & Reichstein, 1949) of **2**. The hydrolyzate, after two days, showed three spots on TLC, two faster moving spots of close mobility and a third spot which had the same mobility as that of D-cymarose. The two faster moving spots, after separation, on alkaline hydrolysis gave D-oleandrose. These results showed that two D-oleandrose units were joined through D-cymarose i.e. D-oleandrose-D-cymarose-D-oleandrose. The ^{13}C NMR spectrum is also in good agreement with the results deduced from chemical reactions and the ^{1}H NMR spectrum. The ^{13}C NMR shifts have been given in Table 1.

The FABMS of **1** showed the cationated molecular ion peaks at m/z 489 and 473 which correspond to $[M+K]^+$ and $[M+Na]^+$. These in turn further fragmented to give the important mass ion fragments. The mass ion fragment at m/z 425 was obtained by the loss of $2CH_3OH$ molecules from m/z 489 which further fragmented to give the fragment ion at m/z 363, 361, 345 and 329 by the usual losses (Khare & Khare, 1987). The loss of three molecules of CH_3OH and one molecule of HCOOH (see Section 3, Experimental) further confirmed the presence of three methoxyl groups and the reducing nature of the trisaccharide. The mass spectrum also showed the characteristic fragments of 2,6-dideoxy sugars reported by Pettit and Brown (Brown, Bruschweiler, Pettit & Teichstein, 1971). In the light of foregoing evidence the structure of **1** was deduced as *O*-β-D-oleandropyranosyl-(1 → 4)-*O*-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranose.

Table 1
^{13}C NMR spectral data for **1** and **3**. (Value (ppm) from internal TMS in $CDCl_3$. cym = D-cymarose, dig = D-digitoxose. ole = D-oleandrose. [a–d] represent assignments in each column that may be interchangeable)

1			**3**		
Sugar	Carbon	Moiety	Sugar	Carbon	Moiety
ole(S_1)	1	91.99	ole(S_1)	1	91.90
	2	31.60[a]		2	36.82[a]
	3	77.31		3	76.99
	4	82.62		4	82.56
	5	71.61		5	67.73
	6	18.23[b]		6	17.95[b]
	-OCH_3	59.01[c]		-OCH_3	58.93[c]
cym(S_2)	1	99.86	dig(S_2)	1	99.54
	2	30.20[a]		2	33.45[a]
	3	76.99		3	75.14
	4	82.42		4	82.39
	5	68.36		5	67.73
	6	22.64[b]		6	18.16[b]
	-OCH_3	56.24[c]			
ole(S_3)	1	101.46	ole(S_3)	1	100.34
	2	31.40[a]		2	35.34[a]
	3	78.39		3	80.45
	4	80.63		4	78.30
	5	75.40		5	71.84
	6	17.96[b]		6	17.86[b]
	-OCH_3	58.26[c]		-OCH_3	56.35[c]

Deniose (**3**) $C_{20}H_{36}O_{10}$, reduced Fehling solution and gave positive xanthydrol (Barton et al., 1952) and Keller–Kiliani (Ngata et al., 1957) reactions along with three secondary methyl group doublets at δ 1.32, 1.26, 1.24 (9H, J = 6 Hz) and three double doublets of one proton each at δ 5.06, 4.91, 4.55 and two singlets of three protons at 3.55 and 3.41 in the ^{1}H NMR spectrum which showed that **3** was a reducing trisaccharide of two 2,6-dideoxy methylated sugars and one 2,6-dideoxy sugar. All the assignments of ^{1}H NMR spectrum were further confirmed by the ^{1}H-^{1}H HOMOCOSY spectrum of **3**.

To identify the sugars of **3**, it was hydrolysed with 0.05 N H_2SO_4 (Rangaswami & Reichstein, 1949), which afforded two chromatographically pure sugars in the ratio of 2:1. The sugars were identified as D-oleandrose (**5**) (Renkonen et al., 1959) and D-digitox-

ose (**7**) (Eppenberger, Kaufmann, Stocklin & Reichstein, 1966) respectively by direct comparison with the authentic samples (PC, TLC, $[\alpha]_D$). Further characterization of the sugars was done by their bromine water oxidation, leading to the formation of their respective lactones, followed by treatment with phenyl hydrazine, which afforded the known D-oleandronic acid phenyl hydrazide mp 133–136°C (Renkonen et al., 1959) and D-digitoxonic acid phenyl hydrazide mp 121–122°C (Eppenberger et al., 1966) (mp, mmp, TLC and PC).

Compound **3** on acetylation with Ac_2O in pyridine gave **4** $C_{26}H_{42}O_{13}$. The ^1H NMR spectrum of **4** at 400 MHz revealed the presence of three acetyl groups as two singlets at δ 2.10 (6H) and 2.00 (3H) which accounted for three acylable hydroxyl groups in **3**. The characteristic downfield shifting (Kumar, Khare & Khare, 1999) of the triplet from δ 3.31 in **3** to 4.64 in **4** suggested that one of the D-oleandrose unit is at the terminal end. Two other downfield shifted protons were found to be H-3′ of D-digitoxose from δ 3.26–3.10 to 5.36 and H-1 of the hexose constituting the reducing end which shifted from δ 5.06 in **3** to 5.96 (dd, J = 9 Hz and 2 Hz) in **4**. The ^1H spectrum of **4** also contained the other ring proton signals.

To identify the sugar at the reducing end **3** was oxidised with Br_2 water followed by hydrolysis with 0.05 N H_2SO_4 which gave three spots on TLC which were separated and identified as D-oleandrose, D-oleandrono-1,4-1actone and D-digitoxose on comparison with authentic samples (TLC, PC). These results led to the conclusion that D-digitoxose unit was flanked on both sides by D-oleandrose. i.e. D-oleandrose-D-digitoxose-D-oleandrose starting from the terminal end.

The configuration of the glycosidic linkages was ascertained by the 400 MHz ^1H NMR spectrum of **3**. The three double doublets of one proton each at δ 5.06 (J = 9 Hz and 2 Hz), 4.91 (J = 9 Hz and 2 Hz) and 4.55 (J = 9 Hz and 2 Hz) were interpreted for three anomeric protons of three sugars. The large coupling constant (9 Hz) of the three anomeric protons were typical of their axial configuration in a $^4C_1$ (D) conformation, suggesting that all the three sugars (including reducing end sugar) have β-configuration (Allgeier, 1968). The structure of **3** was further supported by the data of its ^{13}C NMR spectrum. A relatively upfield appearance of one of the anomeric carbon at δ 91.90 confirmed **3** to be reducing trisaccharide. Besides, the spectrum also confirmed the presence of two methoxy, three secondary methyls and three methylene groups in the distinct regions (see Table 1).

The FABMS of **3** showed the highest mass ion peak at m/z 436 corresponding to M^+. In the higher mass region the prominent mass ion peaks were structurally significant as they could be interpreted as resulting by the losses of H_2O, CH_3OH and CH_3CHO molecules from M^+ in different fragment sequences common to 2,6-dideoxy hexoses (Khare & Khare, 1987). The fragmentation routes are also based on the cleavage of trisaccharide by repeated H-transfer where elimination of terminal sugar less water is visualised to give an ion of the corresponding disaccharide and finally of monosaccharide (Khare & Khare, 1987). The fragment ion peaks in the lower mass region at m/z 145, 131, 127, 113, 95 and 69 could be interpreted to be arising from the characteristic fragmentation pattern of 2,6-dideoxy hexoses reported by Pettit and Brown (Brown et al., 1971). Thus FABMS of **3** completely supported the derived structure. On the basis of the chemical degradation and spectroscopic evidences the structure of **3** was assigned as *O*-β-D-oleandropyranosyl-(1 → 4)-*O*-β-D-digitoxopyranosyl-(1 → 4)-β-D-oleandropyranose.

3. Experimental

General procedures were the same as reported earlier (Deepak, Srivastava & Khare, 1997a). *M. roylei* (10 kg) was extracted as reported earlier Kumar et al., 1999; Shaub, Kaufmann, Stocklin & Reichstein, 1968) yielding hexane (0.81 g), $CHCl_3$ (9.80 g), $CHCl_3$:EtOH(4:1) (4.40 g) and $CHCl_3$:EtOH(3:2) (5.84 g) soluble extract. Royleose (44 mg) was isolated by the repeated column chromatography of $CHCl_3$ extract.

Compound 1, $[\alpha]_D$ +12°(c, 0.34, $CHCl_3$), Found C 56.08. H 8.40 $C_{21}H_{38}O_{10}$ requires C 56.00; H 8.44. ^1H NMR of **1** (400 MHz, $CDCl_3$): δ 5.05 (1H, dd, J = 9 Hz and 2 Hz, H-1), 4.81 (1H, dd, J = 9 Hz and 2 Hz, H-1′), 4.53 (1H, dd, J = 9 Hz and 2 Hz, H-1″), 4.20–4.25 (1H, m, H5′), 4.00–3.95 (1H, m, H-3′), 3.92–3.85 (2H, m, H-5, H-5″), 3.84–3.78 (2H, m, H-3, H-3″), 3.53, 3.45, 3.32 (9H, 3s, 3-OMe), 3.34 (1H, t, J = 9 Hz, H-4), 3.23 (1H, dd, J = 10 Hz and 2 Hz, H-4′), 3.15 (1H, t, J = 9 Hz, H-4″), 2.37–2.32 (1H, m, H-2′eq), 2.22–2,14 (2H, m, H-2eq, H-2″eq), 1.80–1.75 (1H, H-2′ax), 1.68–1.60 (2H, H-2ax, H-2″ax), 1.28, 1.22, 1.20 (9H, 3d, J = 6 Hz, 3-CH_3). FABMS: 489$[M+K]^+$, 473$[M+Na]^+$, 443[489-HCOOH]$^+$, 441[473-CH_3OH]$^+$, 425[489-2CH_3OH]$^+$, 385[443-CH_3CH=CHOH]$^+$, 383[441-CH_3CH=CHOH]$^+$, 363[425-CH_3CHO,-H_2O]$^+$, 361[425-HCOOH,-H_2O]$^+$, 349[M-$C_5H_9O_2$]$^+$, 345[363-H_2O]$^+$, 331[349-H_2O]$^+$, 329[361-CH_3OH]$^+$, 307[383-CH_3CHO,-CH_3OH]$^+$, 307[M-S_2 + H]$^+$, 305[349-CH_3CHO]$^+$, 303[349-HCOOH]$^+$, 271[331-H_3COCHO and 303-CH_3OH], 259[305-HCOOH and 303-CH_3CHO], 206[307-$C_5H_9O_2$]$^+$, 191[206-CH_3]$^+$, 175[206-OCH_3]$^+$, 173[191-H_2O]$^+$, 159[191-CH_3OH]$^+$, 157[175-H_2O]$^+$, 147[191-CH_3CHO]$^+$, 145[S_3^+], 143[175-CH_3OH]$^+$. 129[175-HCOOH]$^+$, 115[159-CH_3CHO and 147-CH_3OH]$^+$,

113[145-CH_3OH]$^+$, 97[129-CH_3OH]$^+$, 95[113-H_2O]$^+$, 69[113-CH_3CHO]$^+$.

3.1. Mild acid hydrolysis of **1**

To a solution of **1** (12 mg) in 1,4 dioxane (1 ml) 0.1 N H_2SO_4 (1 ml) was added and the solution was warmed for 30 min at 50°C. Dioxane was then removed under reduced pressure. The aq. portion was neutralized with freshly prepared $BaCO_3$, filtered and concentrated under reduced pressure followed by column chromatography to afford **5** (6.8 mg, 79%) $[\alpha]_D$ −12° (*c*, 1.2, H_20) and **6** (3.6 mg, 83% $[\alpha]_D$ +53° (*c*, 0.125, H_2O) identified as D-oleandrose and D-cymarose (2:1) by comparison with authentic samples (PC, TLC, $[\alpha]_D$).

3.2. Acetylation of **1**

Compound **1** (15 mg) was acetylated with Ac_2O (2 ml) in pyridine (2 ml) at 100°C for 1 h. The reaction mixture after usual work up gave **2** (15.8 mg, 88%) $[\alpha]_D$ +4° (**c**, 0.76, $CHCl_3$). found C 56.15, H 7.85 requires $C_{25}H_{42}O_{12}$ C 56.18; H 7.86. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.98 (1H, dd, *J* = 9 Hz and 2 Hz, H-1), 4.78 (1H, dd, *J* = 9 Hz and 2 Hz, H-1′), 4.64 (1H, t, *J* = 9 Hz, H-4″), 4.50 (1H, dd, *J* = 9 Hz and 2 Hz, H-1″), 4.08–4.00 (1H, m, H-5′), 3.92–3.83 (3H, m, H-3′, H-5, H-5″), 3.82–3.77 (2H, m, H-3, H-3″), 3.48, 3.46, 3.34 (9H, 3s, 3-OMe), 3.36 (1H, dd, *J* = 10 Hz and 2 Hz, H-4′), 3.25 (1H, t, *J* = 9 Hz, H-4), 2.35–2.30 (1H, m, H-2′eq), 2.22–2.14 (2H, m, H-2eq, H-2″eq), 2.10, 2.00 (6H, 2s, -OCO*CH*$_3$), 1.80–1.75 (1H, H-2′ax), 1.68–1.60 (2H, H-2ax, H-2″ax), 1,32, 1.22, 1.20 (9H, 3d, *J* = 6 Hz, 3-CH_3).

3.3. Very mild acid hydrolysis of **2**

To a solution of **2** (10 mg) in 1,4 dioxane (2 ml) 0.01 N H_2SO_4 (2 ml) was added and the solution was warmed for 30 min. at 50°C. The hydrolysate after usual work up and CC afforded two faster moving spots of close mobility, which on alkaline hydrolysis gave single product i.e. D-oleandrose(**5**)(4.2 mg) $[\alpha]_D$ −12° (*c*, 1.2, H_2O) and a polar spot identified as D-cymarose(**6**)(2.8 mg) $[\alpha]_D$ +53° (*c*, 0.125, H_2O) by comparison with authentic samples (TLC, PC, $[\alpha]_D$).

3.4. Acid phenylhydrazides

Solutions of separated sugars **5** and **6** (6 mg each) in H_2O (0.8 ml) when oxidised with Br_2 water (12 μl) separately using the usual method yielded respective lactones which on treatment with phenylhydrazine yielded known D-oleandronic acid phenylhydrazide (5.8 mg, 65%) mp 133–136°C and D-cymaronic acid phenylhydrazide (5.2 mg, 59%) mp 150–154°C (mp, mmp, TLC and PC).

Deniose (72 mg) was isolated by repeated CC of $CHCl_3$ soluble extract of *M. roylei*. Deniose, $[\alpha]_D$ +25° (*c*, 0.40, $CHCl_3$), found C 55.02; H 8.24 $C_{20}H_{36}O_{10}$ requires C 55.04; H 8.25. It gave blue coloration with vanillin–perchloric acid spray reagent, positive tests in xanthydrol and Keller–Kiliani and reduced Fehling solution. ^{1}H NMR (400 MHz): δ 5.06 (1H, dd, *J* = 9 Hz and 2 Hz, H-1), 4.91 (1H, dd, *J* = 9 Hz and 2 Hz, H-1″), 4.55 (1H, dd, *J* = 9 Hz and 2 Hz, H-1′), 4.25 (1H, m, H-3), 4.19 (1H, m, H-5), 3.89 (1H, m, H-3″), 3.83 (1H, m, H-5″), 3.55, 3.41 (6H, 2s, 2-OMe), 3.40 (1H, m, H-5′)), 3.31 (1H, t, *J* = 9 Hz, H-4″), 3.26–3.10 (3H, m, H-3′, H-4′, H-4), 2.37–2.32 (1H, m, H-2′eq), 2.22–2.10 (2H, m, H-2eq, H-2″eq), 1.82–1.70 (2H, m, H-2, H-2″), 1.54–1.48 (1H, H-2′ax), 1.32, 1.26, 1.24 (9H, 3d, *J* = 6 Hz, 3-CH_3). FABMS: 436[M]$^+$, 419[436-OH]$^+$, 387[419-CH_3OH]$^+$, 369[387-H_2O]$^+$, 355[387-CH_3OH]$^+$, 351[369-H_2O]$^+$, 343[387-CH_3CHO]$^+$, 335[436-$C_5H_9O_2$]$^+$, 329[387-CH_3CH=CHOH]$^+$, 307[351-CH_3CHO]$^+$, 292[436-S_3^+], 289[335-HCOOH]$^+$, 275[307-CH_3OH and 335-H_3CCHO]$^+$, 273[335-H_2O, –CH_3CHO]$^+$, 257[275-H_2O and 289-CH_3OH], 243[275-CH_3OH], 239[257-H_2O], 225[257-CH_3OH], 213[273-H_3COCHO and 257-CH_3CHO]$^+$, 199[243-CH_3CHO]$^+$, 195[239-CH_3CHO]$^+$, 181[213-CH_3OH]$^+$, 137[181-CH_3CHO]$^+$, 131[292-S_2^+], 145[S_3^+], 113[145-CH_3OH and 131-H_2O]$^+$,127[145-H_2O]$^+$, 95[127-CH_3OH and 113-H_2O]$^+$, 69[113-CH_3CHO]$^+$.

3.5. Mild acid hydrolysis of **3**

To a solution of **3** (10 mg) in 1,4 dioxane (1.5 ml) 0.1 N H_2SO_4 (1.5 ml) was added and the solution was warmed for 30 min at 50°C and left at room temperature. The hydrolysate after usual work up and CC gave **5** (6.4 mg, 86%) $[\alpha]_D$ −12°(*c*, 1.1, H_2O) and **7** (2.8 mg, 83%) $[\alpha]_D$ +42.9° (*c*, 0.16, MeOH), in 2:1, identified as D-oleandrose and D-digitoxose respectively by comparison with the authentic samples (TLC, PC, $[\alpha]_D$).

3.6. Bromine water oxidation of **3**

A solution of **3** (10 mg) in water (0.2 ml) was mixed with bromine (2 μl) and shaken in the dark at room temperature for 24 h in a stoppered flask. The excess bromine was then removed under reduced pressure and the acidic reaction mixture was neutralised with freshly precipitated silver carbonate and filtered, H_2S was then passed through the filtrate and again filtered. This filtrate was evaporated to dryness under reduced pressure yielding a dark brown syrupy residue which gave one spot on TLC. This syrup was subjected to

mild acid hydrolysis as usual yielded three spots on TLC and PC, which were identified as D-oleandrose (**5**), D-digitoxose (**7**) and D-oleandrono-1,4-1actone. The identity of lactone was further confirmed by converting into D-oleandronic acid phenyl hydrazide mp 133–136°C (mp, mmp, TLC and PC).

3.7. Acetylation of *3*

Compound **3** (4 mg) was acetylated with Ac_2O (0.5 ml) in pyridine (0.5 ml) at 100°C which after usual work up gave **4** (4.2 mg, 82%) $[\alpha]_D$ +33° (*c*, 0.3, $CHCl_3$), found C 55.49; H 7.46 $C_{26}H_{42}O_{13}$ requires C 55.52, H 7.47. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.96 (1H, dd, J = 9 Hz and 2 Hz, H-1), 5.36 (1H, m, H-3′), 4.87 (1H, dd, J = 9 Hz and 2 Hz, H-1″), 4.64 (1H, t, J = 9 Hz, H-4″), 4.56 (1H, dd, J = 9 Hz and 2 Hz, H-1′), 4.25 (1H, m, H-3), 4.02 (1H, m, H-5), 3.87 (1H, m, H-3″), 3.83 (1H, m, H-5″), 3.46, 3.34 (6H, 2s, 2-OMe), 3.41 (1H, m, H-5′), 3.27 (1H, t, J = 9 Hz, H-4), 3.20 (1H, dd, J = 9 Hz and 2 Hz), 2.37–2.32 (1H, m, H-2′eq), 2.22–2.10 (2H, m, H-2eq, H-2″eq), 2.10, 2.00 (9H, 2s, 3-OCOCH_3), 1.87–1.75 (2H, m, H-2, H-2″), 1.70–1.52 (1H, H-2′ax), 1.25, 1.15 (9H, 2d, J = 6 Hz, 3-CH_3).

3.8. Acid phenylhydrazides

Solutions of separated sugars **5** and **7** (4 mg each) in H_2O when oxidised with Br_2 water separately using the usual method yielded respective lactones which on treatment with phenythydrazine yielded known D-oleandronic acid phenylhydrazide (2.8 mg, 47%) mp 133–136°C and D-digitoxonic acid phenylhydrazide (3.1 mg, 33%) mp 121–122°C (mp, mmp, TLC and PC).

Acknowledgements

Authors are thankful to the University Grants Commission, New Delhi for financial assistance and Regional Sophisticated Instrumentation Centre, Central Drug Research Institute, Lucknow for successful running of NMR and Mass spectra.

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