Phytochemistry, Vol 27, No 4, pp 1173-1179, 1988
Printed in Great Britain

0031-9422/88 $3 00+0 00

PREGNANE GLYCOSIDES FROM AN ANTITUMOUR FRACTION OF *PERIPLOCA SEPIUM*

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(*Received* 5 *August* 1987)

Key Word Index—*Periploca sepium*, Asclepiadaceae, pregnane glycoside, Δ^5-pregnene-3β,16β,20(*R*)-triol, Δ^5-pregnene-3β,16α,20(*S*)-triol

Abstract—Three new pregnane glycosides (S-4a, S-5 and S-10) and two known pregnane glycosides (S-4b and S-6) were isolated from an antitumour fraction of the root barks of *Periploca sepium*. Their structures were determined by ^{1}H and ^{13}C NMR, FABMS and SIMS spectroscopy and some chemical transformations One of the glycosides (S-4a) contained the new aglycone Δ^5-pregnene-3β,16β,20(*R*)-triol

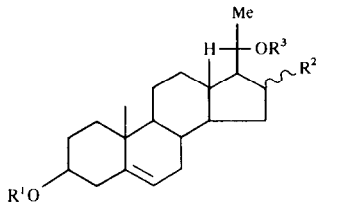
INTRODUCTION

In two earlier papers [1, 2], we reported on the isolation of eight substances (S-1–S-8), four of which we characterized, from the antitumour fraction prepared by subjecting the chloroform extract of *Periploca sepium* The present paper deals mainly with the separation and characterization of three new pregnane glycosides (S-4a, S-5 and S-10) and two known glycosides (S-4b and S-6)

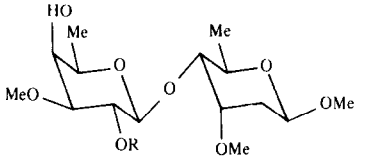
RESULTS AND DISCUSSION

The mixture of pregnane glycosides designated S-4 gave S-4a (**1**), S-4b (**2**) and S-10 **1** and **2** showed the same molecular ion peak as a cationized cluster ion $[M(C_{56}H_{92}O_{25})+Na]^+$ at *m/z* 1187 and $[M+K]^+$ at *m/z* 1203 on FABMS On acid hydrolysis with 1 M H_2SO_4 in 50% aqueous methanol, **1** and **2** yielded the aglycones **8** and **12** respectively, and, as shown by TLC, the sugars D-cymarose, D-digitalose and D-glucose

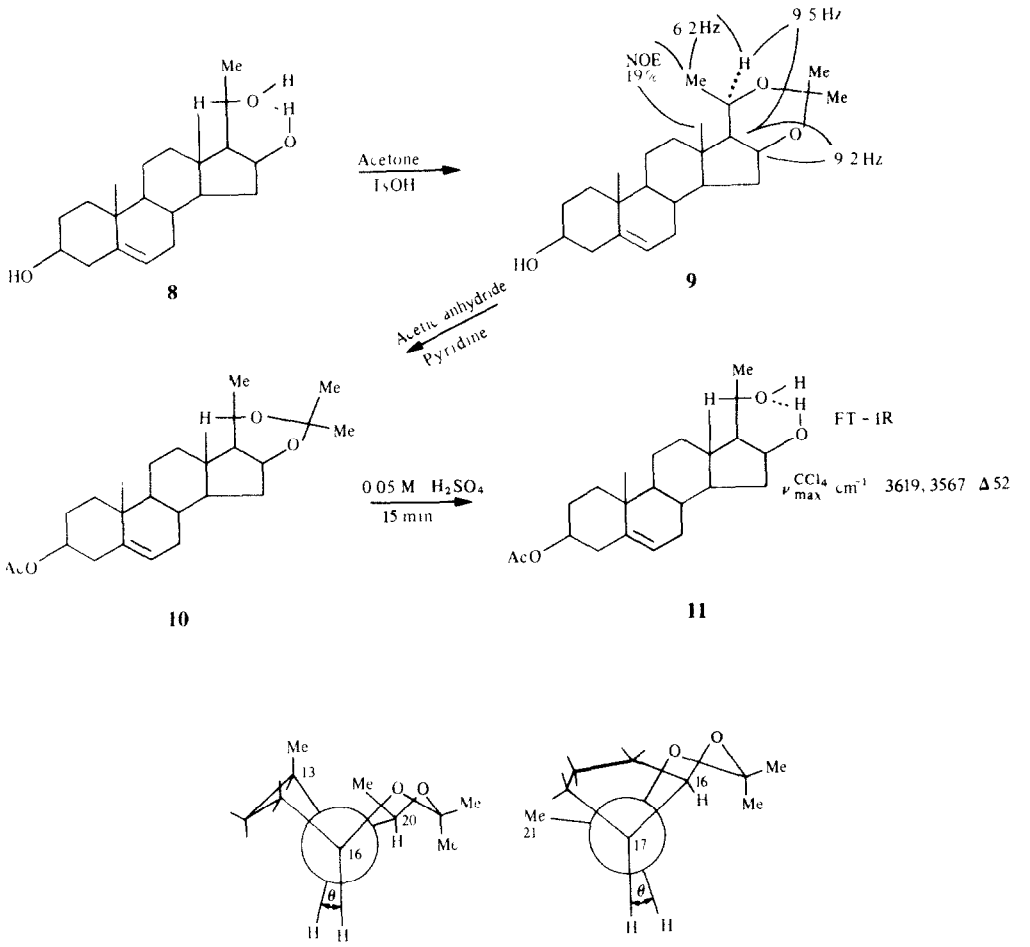
8 exhibited a molecular ion peak at *m/z* 334 [M^+, $C_{21}H_{34}O_3$) in its EIMS Comparison of the ^{1}H and ^{13}C NMR and MS data with those of Δ^5-pregnene-3β,20(*S*)-diol [1], indicated that **8** had one additional hydroxyl group at C-16 [δ72 92 (*d*) and δ4 50, *ddd*, *J* =7.8, 5.5 and 2 3 Hz]. In spite of the absence of a hydroxyl group at either C-15 or C-17 an acetonide (**9**) of **8** was easily prepared between the two hydroxyl groups at C-16 and C-20. Decoupling of the signals at δ1 20 (Me-21), 3 85 (H-20) and 4 16 (H-16) of **9** revealed that the coupling constants were $J_{17,20}$=9 5 Hz and $J_{16,17}$ =9.2 Hz Also, when the doublet signal at δ1.31 (Me-21) was irradiated, a NOE was observed with a 19% enhancement of the intensity due to the signal at δ0 89 (Me-18). On the basis of the above result, both dihedral angles (θ) between H-20 and H-17, and H-17 and H-16 were confirmed to be about 22° as shown in Fig 2. Consequently, the absolute configurations of C-20 and C-16 were assigned as '*R*' and 'β', respectively, and the structure of **8** was established as Δ^5-pregnene-3β,16β,20(*R*)-triol which has been isolated for the first



	R^1	R^2	R^3
1	cym (4-1) 2-O-Ac-dig	—OH	dig (2-1) glc (6-1) glc
2	cym (4-1) 2-O-Ac-dig	···OH	dig (2-1) glc (6-1) glc
3	cym (4-1) dig	—H	dig (2-1) glc (6-1) glc
4	cym (4-1) 2-O-Ac-dig	—H	dig (2-1) glc (6-1) glc
5	H	—OH	dig (2-1) glc (6-1) glc
6	H	·····OH	dig (2-1) glc (6-1) glc
7	H	—H	dig (2-1) glc (6-1) glc
8	H	—OH	H
12	H	·····OH	H



13 R = H
14 R = Ac



time In addition, the molecular model showed that the two hydroxyl groups at C-16 and C-20 of **8** lie close together, and it was presumed that they formed a hydrogen bond favourable for stability. In order to confirm the existence of this bond, **9** was acetylated to afford **10**, and then **10** was hydrolysed with 0 005 M H_2SO_4 in aqueous MeOH to yield **11** The FT-IR spectrum of **11** (0 003 M in CCl_4) showed two absorption bands due to a free hydroxyl group and an intramolecular hydrogen bond (3619 and 3567 cm^{-1}) The length of the hydrogen bond was calculated as 1 98 Å from the empirical equation presented by Kuhn [3, 4]

12 showed a molecular ion peak at *m/z* 334 (M^+, $C_{21}H_{34}O_3$) in its EIMS Its ^1H and ^{13}C NMR and MS spectral data were similar to those of **8**, but the NMR spectral data due to C-16 (δ77 62, *d* and δ4 35, *ddd*, *J* =7 8, 6 8 and 1 8 Hz) and C-20 (δ70 48 and 3 91, *br q*, *J* =6 2 Hz) of **12** were different from those of **8** Because the acetonide of **12** involving the two hydroxyl groups at C-16 and C-20 could not be prepared in the usual way, the configuration of C-16 was deduced to be 'α' From the above results, **12** had to be Δ^5-pregnene-3β,16α,20(*S*)-triol This was confirmed by comparing the recorded ^1H NMR spectrum with the reported one for this compound [5, 6]

The ^1H and ^{13}C NMR spectral data of **1** and **2** showed the presence of five anomeric proton and carbon signals in the sugar moiety, respectively Upon partial acid hydrolysis with 0 025 M H_2SO_4 in 50% aqueous MeOH, both **1** and **2** gave **14** which had already been shown to be methyl 2-*O*-acetyl-β-D-digitalopyranosyl(1→4)-β-D-cymaropyranoside by direct comparison with an authentic sample [2], along with **5** from **1** and **6** from **2** Also, the physical and spectral data of **5** were identical with those of S-10 Since the ^{13}C chemical shifts of **5** and **6** at C-3 were shifted upfield (−6 15 and −6 27 ppm) in comparison to those of **1** and **2**, **14** was linked to the C-3 hydroxyl group of **5** and **6**. **5** and **6** were further hydrolysed with 1 M H_2SO_4 in 50% aqueous methanol to furnish the aglycones **8** and **12**, respectively, and, as shown by TLC, D-digitalose and D-glucose From the anomeric proton and carbon signals in the NMR spectra, **5** and **6** consisted of 1 mol of the aglycone and D-digitalose (δ104 03, *d*, and 104.01, *d*), δ4.62, *d*, *J*=8 0 Hz and δ4 72, *d*, *J*=7 84 Hz) and 2 mol of D-glucose (δ104 28, *d*, 104.99, *d* and 105.29, *d*, 105 48, *d*, δ5 38, 5 47, *d*, *J*=7 8 Hz and δ5 13, 5 36, *d*, *J*=7 7 Hz, respectively) and these sugars were joined by β-glycosyl linkage as can be seen from their coupling constants in Table 1

Both acetates of **5** and **6**, which were prepared with

acetic anhydride–pyridine in the usual way, in the EIMS exhibited three fragment ion peaks due to *O*-acetylated sugars at *m/z* 821 $[glc(Ac)_4\text{-}glc(Ac)_3\text{-}dig(Ac)]^+$, 619 $[glc(Ac)_4\text{-}glc(Ac)_3]^+$, 331 $[glc(Ac)_4]^+$ which revealed the sequence of the sugar moiety The glycosylation shifts [7] of **5** and **6** were observed at C-20 (+12 68 and +11.52 ppm), C-6 of one glucose (+6 78 and +4 32 ppm) [8] and C-2 of digitalose (+4.83 and +5.03 ppm) [9] as shown by comparison of these signals with the corresponding ones in their aglycones, methyl glycopyranoside and methyl digitalopyranoside Consequently, **5** and **6** were determined to be Δ^5-pregnene-3β,16β,20(*R*)-triol 20-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl(1→2)-β-D-digitalopyranoside and Δ^5-pregnene-3β,16α,20(*S*)-triol 20-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl (1→2)-β-D-digitalopyranoside, respectively. Further, based upon the above results, **1** was established as Δ^5-pregnene-3β,16β,20(*R*)-triol 3-*O*-[2,-*O*-acetyl-β-D-digitalopyranosyl (1→4)-β-D-cymaropyranoside] 20-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl (1→2)-β-D-digitalopyranoside] which is a new pregnene glycoside, and **2** as Δ^5-pregnene-3β,16α,20(*S*)-triol 3-*O*-[2-*O*-acetyl-β-D-digitalopyranosyl (1→4)-β-D-cymaropyranoside] 20-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl (1→2)-β-D-digitalopyranoside] [10].

S-5 (**3**) and S-6 (**4**) gave molecular ion peaks as cationized cluster ions at *m/z* 1124 $[M(C_{54}H_{90}O_{23})+NH_4]^+$ and 1166 $[M(C_{56}H_{92}O_{24})+NH_4]^+$, respectively, on FABMS. As described in the previous paper [1], **3** and **4** were hydrolysed with acid to yield the same aglycone which was confirmed as Δ^5-pregnene-3β,20(*S*)-diol, and, as indicated by TLC, D-cymarose, D-digitalose and D-glucose. The ^1H and ^{13}C NMR spectral data of **3** and **4** showed five anomeric proton and carbon signals due to the sugar moiety, and were very similar except for a methyl signal due to the acetyl group of **4** **4** was hydrolysed with 0.2 M NaOH to yield **3**. Upon above partial acid hydrolysis, both **3** and **4** gave **7** along with **13** from **3** and 2-*O*-acetyl-β-D-digitalopyranosyl (1→4)-β-D-cymaropyranoside (**14**) from **4** Since deacetylation of **14** with 0.2 M NaOH provided **13**, the structure of **13** was deduced to be methyl β-D-digitalopyranosyl(1→4)-β-D-cymaropyranoside.

7 was further hydrolysed with 1 M H_2SO_4 in 50% aqueous MeOH to give Δ^5-pregnene-3β,20(*S*)-diol, D-digitalose and D-glucose. Anomeric signals in the ^1H and ^{13}C NMR spectra showed the presence of 1 mol each of the aglycone and digitalose (δ104.19, *d*, and δ4 7, *d*, *J* = 7.8 Hz), and 2 mol of glucose (δ104 40, *d*, 105 17 *d*, and δ5.26, *d*, *J* = 7.6 Hz; 5.37, *d*, *J* = 7.8 Hz). The acetate of **7** in the EIMS showed the same three fragment ion peaks at *m/z* 821, 619 and 331 as shown by the acetates of **5** and **6**. Therefore, **7** was deduced to be Δ^5-pregnene-3β,20(*S*)-diol 20-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl (1→2)-β-D-digitalopyranoside, whose structure was also supported from the ^{13}C NMR spectral data On the basis of above observation, **3** was deduced to be Δ^5-pregnene-3β,20 (*S*)-diol 3-*O*-[β-D-digitalopyranosyl (1→4)β-D-cymaropyranoside]20-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl(1→2)-β-D-digitalopyranoside] which is obtained from a natural source for the first time, and **4** as Δ^5-pregnene-3β,20 (*S*)-diol 3-*O*-[2-*O*-acetyl-β-D-digitalopyranosyl (1→4)-β-D-cymaropyranoside] 20-*O*-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl(1→2)-β-D-digitalopyranoside] [11].

EXPERIMENTAL

General procedures Mps uncorr, ^1H and ^{13}C NMR: 400 and 100 6 MHz respectively with TMS as int standard, FABMS JEOL JMS DX-303, SIMS and EIMS Hitachi M-80 mass spectrometer

The following solvent systems were used for Kieselgel $60F_{254}$ (Merck) and ODS (Fujigel) TLC· solvent I $CHCl_3$–MeOH (24 1), II $CHCl_3$–MeOH–H_2O (7 3·0 5), III $CHCl_3$–MeOH–H_2O (25 17 3), IV MeOH–H_2O (3:2) Detection 10% H_2SO_4 spray and heating

Plant material The root barks of *Periploca sepium* used in this experiment were purchased in China A small number of them are kept in our laboratory The material was identified as *P sepium* by Dr M Satake, Botanical garden director at Tsukuba Medicinal Plant Research Station, National Institute of Hygienic Science, Japan.

Extraction and isolation Extraction and isolation of S-4, S-5 (**3**) and S-6 (**4**) were described in a previous paper [1] S-4 (387 mg) was subjected to RP-18 CC with MeOH–H_2O (3 2) to furnish S-4a (**1**, 147 mg) and S-4b (**2**, 161 mg) Repeated RP-8 (with MeOH–H_2O, 7 3) and RP-18 (with MeOH–H_2O, (3 2)) CC of the $CHCl_3$–MeOH (5.1) fraction obtained in an earlier study [1] gave S-10 (**5**, 3 5 mg) **1**, white powder, mp 182–184°, $[\alpha]_D^{20}$ −16 24° (MeOH, *c* 12), FABMS *m/z* 1187 $[M(C_{56}H_{92}O_{25})+Na]^+$ and 1203 $[M+K]^+$ **2**, white powder, mp 185–187°, $[\alpha]_D^{20}$ −27 16° (MeOH, *c* 0 3), FABMS *m/z*, 1187 $[M(C_{56}H_{92}O_{25})+Na]^+$ and 1203 $[M+K]^+$ **3**, white powder, mp 175–177°, $[\alpha]_D^{20}$ −25 22° (EtOH; *c* 1 4), SIMS *m/z*. 1124 $[M(C_{54}H_{90}O_{27})+NH_4]^+$. **4**, white powder, mp 181–183°, $[\alpha]_D^{20}$ −22.90° (EtOH, *c* 0 4), SIMS *m/z* 1148 $[M(C_{56}H_{92}O_{24})+NH_4]^+$ **5**, white powder, mp 167–169°, $[\alpha]_D^{20}$ −2 6° (MeOH; *c* 0 2), FABMS *m/z* 819 $[M(C_{30}H_{24}O_{23})+H]^+$, 842 $[M+Na+H]^+$ and 857 $[M+K]^+$

Partial acid hydrolysis of **1**–**4** Each sample (50 mg) was hydrolysed with 0 025 M H_2SO_4 in 50% aq MeOH (3 ml) under reflux for 20 min at 80° The reaction mixture was diluted with H_2O (10 ml) and the MeOH evapd *in vacuo* at room temp The residue was partitioned between H_2O and $CHCl_3$ The $CHCl_3$ extract was concentrated and chromatographed on silica gel with EtOAc to give **13** from **3**, and **14** from **1**, **2** and **4** The aq layer was extracted with *n*-BuOH, and then the BuOH extract was purified by means of RP-18 CC with solvent IV to give **5** (21.5 mg) from **1**, **6** (23 mg) from **2**, **7** (21 mg) from **3** and **4** respectively

5 was identical with S-10 **6**, white powder, mp 179– 182°, $[\alpha]_D^{20}$ −38 68° (MeOH, *c* 0 2), FABMS *m/z* 819 $[M(C_{30}H_{24}O_{23})+H]^+$, 842 $[M+Na+H]^+$ and 857 $[M+K]^+$ **7**, white powder, mp 238–240°, $[\alpha]_D^{20}$ −27 5° (MeOH, *c* 0 2). **13**, colourless needles, mp 115–117°, $[\alpha]_D^{20}$ +18 18° ($CHCl_3$, *c* 0.1) ^1H NMR ($CDCl_3$)· δ1 32 (3H, *d*, *J* = 6 3 Hz, cym-6), 1 35 (3H, *d*, *J* = 6 5 Hz, dig-6), 1 59 (1H, *dq*, *J* = 13 5, 9 1 Hz, cym-2a), 2 16 (1H, *ddd*, *J* = 13.5, 6.2, 2.2 Hz, cym-2b), 3 20 (1H, *dd*, *J* = 9 5, 3 4 Hz, dig-3), 3.34 (1H, *dd*, *J* = 9 2, 3.0 Hz, cym-4), 3 44, 3 47 (3H, *s*, respectively, cym-1 and 3-OMe), 3 52 (3H, *s*, dig-3-OMe), 3 59 (1H, *dd*, *J* = 6 5, 1 1 Hz, dig-5), 3 71 (1H, *dd*, *J* = 9 5, 7 9 Hz, dig-2), 3 81 (1H, *dd*, *J* = 6 0, 3 0 Hz, cym-3), 3 84 (1H, *dd*, *J* = 3 4, 1 1 Hz, dig-4), 3 97 (1H, *dd*, *J* = 9 2, 6 3 Hz, cym-5), 4 30 (1H, *d*, *J* = 7 9 Hz, dig-1), 4 66 (1H, *dd*, *J* = 9 1, 2 2 Hz, cym-1)

14 is described in a previous paper [2] *Deacetylation of* **4** *and* **14** A soln of either **4** (10 mg) or **14** (4 6 mg) in 0.2 M NaOH (1 5 ml) was warmed at 50° for 2 hr with stirring under N_2 gas The reaction mixture was neutralized with Amberlite IR-120 and concentrated *in vacuo* to give the crude product **3** from **4** and colourless needles of **13** from **14** The product **3** was purified by RP-18 CC with solvent III

Table 1 ^{1}H NMR spectral data for S-4a (**1**), S-4b (**2**), S-5 (**3**), S-6 (**4**)

H	**8** ($CDCl_3$)	**5**	**1**	**12** ($CDCl_3$)
3 H	3 51 *m*			3 53 *m*
6 H	5 35 *m*	5 33 *m*	5 33 *m*	5 35 *m*
16 H	4 50 *ddd* (*J* = 7 8, 5 5, 2 3 Hz)			4 35 *ddd* (*J* = 7 8, 6 8, 2 2 Hz)
18 Me	0 89 *s*	1 04 *s*	0 96 *s*	0 69 *s*
19 Me	1 02 *s*	1 04 *s*	1 01 *s*	1 00 *s*
20 H	4 13 *dq* (*J* = 6 2, 3 6 Hz)	4 25 *br*	4 25 *br*	3 91 *br q* (*J* = 6 2 Hz)
21 Me	1 31 *d* (*J* = 6 2 Hz)	1 67 *d* (*J* = 6 2 Hz)	1 67 *d* (*J* = 6 1 Hz)	1 27 *d* (*J* = 6 2 Hz)
3 cym				
1 H			5 26 *dd* (*J* = 9 6, 1 7 Hz)	
6 Me			1 47 *d* (*J* = 6 3 Hz)	
OMe			3 41 *s*	
dig				
1-H			4 73 *d* (*J* = 8 0 Hz)	
2-H			5 84 *dd* (*J* = 8 0, 10 Hz)	
6 Me			1 57 *d* (*J* = 6 4 Hz)	
OMe			3 53 *s*	
20 dig				
1 H		4 62 *d* (*J* = 8 0 Hz)	4 63 *d* (*J* = 8 0 Hz)	
2 H		4 92 *dd* (*J* = 8 0 9 7 Hz)	4 93 *dd* (*J* = 8 0, 9 6 Hz)	
6 Me		1 46 *d* (*J* = 6 3 Hz)	1 45 *d* (*J* = 6 3 Hz)	
OMe		3 52 *s*	3 52 *s*	
glc				
1 H		5 38 *d* (*J* = 7 8 Hz)	5 37 *d* (*J* = 7 8 Hz)	
glc'				
1 H		5 47 *d* (*J* = 7 8 Hz)	5 46 *d* (*J* = 7 8 Hz)	

Acetylation of **5**, **6** *and* **7** was performed with Ac_2O–C_5H_5N (12 hr, room temp) in the usual way The EIMS of each acetate showed characteristic fragments at *m/z* 821, 619, 331

Acid hydrolysis of **5**, **6** *and* **7** Each sample (20 mg) was refluxed with 1 5 M H_2SO_4 in 50% aq MeOH (2 ml) for 1 5 hr on a water bath The reaction mixture was diluted with H_2O (10 ml) and extracted with $CHCl_3$ The $CHCl_3$ extract was purified by silica gel CC with solvent I to afford about 6 mg of compounds **8**, **12** and Δ^5-pregnene-3β,20(*S*)-diol [1], respectively The aq layer was neutralized with Amberlite IRA-94 and evapd to dryness *in vacuo* Each sample showed the presence of D-digitalose and D-glucose on silica gel TLC (solvent II and III)

8, white powder, 217-219°, $[\alpha]_D^{20}$ −15 79° (EtOH, *c* 0 1), EIMS *m/z* 334 [M^+, $C_{21}H_{34}O_3$] **12**, white powder, 222–224°, $[\alpha]_D^{20}$ −60 2° (MeOH, *c* 0 1), EIMS *m/z* 334 [M^+, $C_{21}H_{34}O_3$]

Acetonide formation from **8** *p*-TsOH (5 mg) was added to a soln of **8** (15 mg) in Me_2CO (3 ml) and the mixture refluxed overnight The reaction mixture was poured into H_2O and extracted with Et_2O The Et_2O layer was washed with satd $NaHCO_3$ soln, dried with dry Na_2SO_4 and concd to yield **9** as a solid ^{1}H NMR ($CDCl_3$) δ0 90 (3H, *s*, Me-18), 1 03 (3H, *s*, Me-19), 1 20 (3H, *d*, *J* = 6 2 Hz, Me-21), 3 51 (1H, *m*, H-3), 3 85 (1H, *dq*, *J* = 9 5, 6 2 Hz, H-20), 4 16 (1H, *ddd*, *J* = 9 5, 9 2, 1 8 Hz, H-16), 5 36 (1H, *m*, H-6), 1 32 (6H, *s*, >C(Me)$_2$), ^{13}C NMR ($CDCl_3$) δ37 30 (*t*, C-1), 32 14 (*t*, C-2), 71 78 (*d*, C-3), 42 35 (*t*, C-4), 141 95 (*s*, C-5), 121 42 (*d*, C-6) 31 70 (*t*, C-7), 31 14 (*d*, C-8), 50 22 (*d*, C-9),

and related compounds (400 MHz, C_5D_5N, TMS as int standard)

6	2	7	3	4
5 27 *m*	5 24 *m*	5 40 *m*	5 35 *m*	5.36 *m*
0.67 *s*	0 64 *s*	0.67 *s*	0 64 *s*	0 66 *s*
1 03 *s*	0.96 *s*	1 02 *s*	0 93 *s*	0 95 *s*
3 83 *br*	3 80 *br*	3 75 *br q*	3 76 *br q*	3 76 *br q*
		(*J* = 6.2 Hz)	(*J* = 6 3 Hz)	(*J* = 6 5 Hz)
1 61 *d*	1 59 *d*	1 61 *d*	1 58 *d*	1 60 *d*
(*J* = 6 0 Hz)	(*J* = 6 5 Hz)	(*J* = 6 2 Hz)	(*J* = 6 3 Hz)	(*J* = 6 5 Hz)
	5 26 *dd*		5.29 *dd*	5 28 *dd*
	(*J* = 9.6, 1 6 Hz)		(*J* = 9.6, 1 7 Hz)	(*J* = 9 6, 1.6 Hz)
	1.46 *d*		1 56 *d*	1 47 *d*
	(*J* = 6 3 Hz)		(*J* = 6 3 Hz)	(*J* = 6 25 Hz)
	3.42 *s*		3 50 *s*	3 42 *s*
	4.76 *d*		4 72 *d*	4 74 *d*
	(*J* = 8 0 Hz)		(*J* = 7 8 Hz)	(*J* = 8 0 Hz)
	5 83 *dd*			5 84 *dd*
	(*J* = 8 0, 10 Hz)			(*J* = 8.0, 10 Hz)
	1 57 *d*		1 65 *d*	1.58 *d*
	(*J* = 6 5 Hz)		(*J* = 6 3 Hz)	(*J* = 6 5 Hz)
	3 53 *s*		3 55 *s*	3 53 *s*
4 72 *d*	4 74 *d*	4 69 *d*	4.67 *d*	4 69 *d*
(*J* = 7.8 Hz)	(*J* = 7 5 Hz)	(*J* = 7 8 Hz)	(*J* = 7.8 Hz)	(*J* = 7 8 Hz)
4 78 *br*	4 78 *br*	4 86 *dd*	4.84 *dd*	4 87 *dd*
		(*J* = 7 8, 9.7 Hz)	(*J* = 7.8, 9 6 Hz)	(*J* = 7 9, 9 7 Hz)
1 53 *d*	1 53 *d*	1.49 *d*	1 47 *d*	1 49 *d*
(*J* = 6 3 Hz)	(*J* = 6 3 Hz)	(*J* = 6 4 Hz)	(*J* = 6 4 Hz)	(*J* = 6 4 Hz)
3 58 *s*	3 59 *s*	3 51 *s*	3 56 *s*	3 52 *s*
5 13 *d*	5 11 *d*	5 26 *d*	5 25 *d*	5 27 *d*
(*J* = 7 7 Hz)	(*J* = 7 7 Hz)	(*J* = 7 6 Hz)	(*J* = 7 8 Hz)	(*J* = 7 6 Hz)
5 36 *d*	5 35 *d*	5 37 *d*	5 36 *d*	5 37 *d*
(*J* = 7 6 Hz)	(*J* = 7 6 Hz)	(*J* = 7 8 Hz)	(*J* = 7 8 Hz)	(*J* = 7 8 Hz)

36 78 (*s*, C-10), 20 61 (*t*, C-11), 39 73 (*t*, C-12), 41 52 (*s*, C-13), 52 89 (*d*, C-14), 37 30 (*t*, C-15), 70 66 (*d*, C-16), 64.48 (*d*, C-17), 14 88 (*q*, C-18), 19 46 (*q*, C-19), 77 62 (*d*, C-20), 21 48 (*q*, C-21), 24 01, 24 98 (*q*, >C(Me)$_2$, respectively)

Acetylation of **9** Acetylation of **9** (5 mg) with Ac_2O–C_5H_5N was carried out in the usual way to furnish **10** ^{13}C NMR ($CDCl_3$) δ 37 02 (*t*, C-1), 29 72 (*t*, C-2), 73 90 (*d*, C-3), 38 13 (*t*, C-4), 140 90 (*s*, C-5), 122 33 (*d*, C-6), 32 10 (*t*, C-7), 30 93 (*d*, C-8), 50 11 (*d*, C-9), 36 74 (*s*, C-10), 20 54 (*t*, C-11), 39 65 (*t*, C-12), 40 71 (*s*, C-13), 52 79 (*d*, C-14), 32 91 (*t*, C-15), 70.63 (*d*, C-16), 60 79 (*d*, C-17), 14 85 (*q*, C-18), 19 35 (*q*, C-19), 64 45 (*d*, C-20), 21 46 (*q*, C-21), 23 98, 24 95 (*q*, >C(Me)$_2$), 99.81 (*s*, >C(Me)$_2$), 21 46 (*q*, Ac-Me), 170 38 (*s*, Ac-CO)

Preparation of **11** *from* **10** **10** (5 mg) was refluxed with 0 05 M H_2SO_4 in 50% aq MeOH (1 ml) for 14 min at 60°. The reaction mixture was treated as usual way to yield **11** ^{1}H NMR ($CDCl_3$) δ0.89 (3H, *s*, Me-18), 1 03 (3H, *s*, Me-19), 1 31 (3H, *d*, *J* = 6 2 Hz, Me-21), 2 03 (3H, *s*, Ac), 4 13 (1H, *br q*, *J* = 6.2 Hz, H-20, 4 50 (1H, *ddd*, *J* = 7 8, 5.5, 2 2 Hz, H-16), 4 59 (1H, *m*, H-3), 5 38 (1H, *m*, H-6), ^{13}C NMR ($CDCl_3$) δ36.98 (*t*, C-1), 29 72 (*t*, C-2), 73 91 (*d*, C-3), 38 12 (*t*, C-4), 141 95 (*s*, C-5), 122 33 (*d*, C-6), 31 87 (*t*, C-7), 31.09 (*d*, C-8), 50 24 (*d*, C-9), 35 62 (*s*, C-10), 20.53 (*t*, C-11), 39 22 (*t*, C-12), 41 10 (*s*, C-13), 54 24 (*d*, C-14), 35 62 (*t*, C-15), 73 19 (*d*, C-16), 63 04 (*d*, C-17), 13 96 (*q*, C-18), 19 32 (*q*, C-19), 66 69 (*d*, C-20), 23 64 (*q*, C-21), 21 42 (*q*, Ac-Me), 170 55 (*s*, Ac-CO)

Table 2 ^{13}C NMR spectral data for aglycone moieties of S-4a (**1**), S-4b (**2**), S-5 (**3**), S-6 (**4**) and related compounds (100 6 MHz, C_5H_5N, TMS as int standard)

C	**8**	**8** ($CDCl_3$)	**5**	**1**	**12**	**12** ($CDCl_3$)	**6**	**2**	**7**	**3**	**4**
1	37 77	37 26	37 72	37 37	37 76	37 17	37 65	37 35	37 76	37 50	37 45
2	32 21	31 69	32 29	30 34	32 18	31 24	32 16	30 35	32 61	30 37	30 33
3	71 25	71 79	71 28	77 43	71 26	71 74	71 20	77 47	71 23	77 79	77 78
4	43 48	42 33	43 50	39 35	43 54	42 31	43 49	39 37	43 47	39 41	39 32
5	142 00	140 88	141 77	140 65	142 06	140 76	141 60	140 51	141 77	140 74	140 67
6	121 07	121 43	121 33	122 01	121 12	121 51	121 48	122 22	121 31	121 98	122 06
7	32 61	31 90	32 65	32 20	32 66	31 65	32 64	32 11	32 17	32 14	32 14
8	31 46	31 15	31 73	31 56	31 56	31 65	31 50	31 36	31 92	31 83	31 60
9	50 56	50 16	50 37	50 25	50 60	50 06	49 99	49 88	50 42	50 36	50 31
10	37 77	36 60	36 93	36 89	37 00	36 45	36 88	36 89	36 86	36.90	36 68
11	20 92	20 62	20 98	20 85	20 76	20 40	20 92	20 85	21 12	21 05	21 04
12	39 57	39 30	39 74	39 62	39 23	38 78	39 43	39 37	39 15	39 07	39 05
13	41 30	41 12	41 28	41 18	42 68	42 40	42 95	42 37	42 55	41 52	41 50
14	54 59	54 33	54 41	54 27	54 54	54 01	53 64	53 54	58 07	58 06	58 03
15	36 51	35 67	35 70	35 62	35 67	34 71	35 82	35 07	26 92	26 86	26 90
16	72 92	73 23	70 96	70 90	77 05	77 62	77 27	77 23	24 42	24 45	24 45
17	63 74	63 07	62 79	62 70	68 24	67 48	68 48	68 46	56 63	56 63	56 58
18	14 23	13 98	13 71	13 62	13 97	13 78	13 96	13 89	12 63	12 62	12 60
19	19 57	19 44	19 59	19 37	19 62	19 42	19 60	19 43	19 56	19 40	19 40
20	65 93	66 71	79 39	79 37	69 42	70 48	82 00	81 94	81 68	81 72	81 71
21	24 07	23 65	22 16	22 13	24 49	23 95	23 75	23 71	23 17	23 19	23 20

Table 3 ^{13}C NMR spectral data for sugar moieties of S-4a (**1**), S-4b (**2**), S-5 (**3**), S-6 (**4**) and related compounds (100 6 MHz C_5H_5N, TMS as int standard)

C	**5**	**1**	**6**	**2**	**7**	**3**	**4**	**13** ($CDCl_3$)	**14**
3 cym									
1		96 30		96.24		96 22	96 23	98 98	99 00
2		37 14		37 19		37 14	37 12	35 44	35 84
3		77 43		77 31		77 41	77 36	76 37	77 49
4		84 21		84 22		83 51	84 21	83 66	82 90
5		68 80		68 84		70 66	68 78	68 21	69 11
6		18 51		18 54		18 81	18 49	17 88	18 27
OMe		58 62		58 68		58 63	58 62	58 31	58 07
dig									
1		103 56		103 56		106 80	103 56	102 47	106 39
2		71 48		71 58		70 84	71 40	70 88	70 46
3		82 30		82 33		84 79	82 27	81 56	84 45
4		67 86		68 46		69 41	67 79	68 00	68 07
5		71 42		71 46		71 17	71 40	70 34	70 83
6		17 12		17 16		17 30	17 13	16 39	16 94
OMe		56 52		56 58		57 23	56 49	57 34	56 87
OAc		169 67		169 71			169 68	169 37	
		21.10		21 15			21 11	20 88	
20 dig									
1	104 03	104 01	104 01	104 01	104 19	104 18	104 20		
2	75 53	75 56	75 73	75 72	76 21	76 21	76 22		
3	85 48	85 42	85 67	85 64	85 38	85 39	85 38		
4	68 54	68 45	69 08	69 07	68 36	68 43	68 34		
5	71 56	71 76	71 60	71 58	72 19	72 20	72 16		
6	17 36	17 31	17 28	17 26	17 32	17 30	17 33		
OMe	56 75	56 70	57 15	57 16	56 67	57 23	56 63		

Table 3 *continued*

C	5	1	6	2	7	3	4	13 ($CDCl_3$)	14
glc									
1	104 28	104 27	105.29	105 29	104 40	104 40	104 44		
2	75 16	75 12	74 51	74 50	75 23	75 24	75.21		
3	77 73	77 70	77 27	77 31	77 77	77 76	77 78		
4	72 10	72 01	70 11	70.11	71 23	71 17	71 40		
5	77.66	77 57	76 80	76 74	77 28	77 41	77 28		
6	69 78	69 75	67 32	67 24	69 95	69 98	69 95		
glc									
1	104 99	104.97	105 48	105 48	105 17	105 18	105 20		
2	74.95	74 97	74 97	74 95	75 45	75 45	75 48		
3	78 29	78 28	78 25	78 23	78 19	78 19	78 22		
4	72 10	72 01	71 05	71 03	71 90	71 90	71 88		
5	78 65	78 59	78.56	78 54	78 19	78 19	78 22		
6	63 04	62 99	62 74	62 72	62 94	62 95	62 92		

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