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FURTHER POLYOXYPREGNANES FROM *MARSDENIA TENACISSIMA**

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Key Word Index—*Marsdenia tenacissima*; Asclepiadaceae; polyoxypregnanes; structural elucidation; marstenacigenins A and B.

Abstract—Two new polyoxypregnanes, designated marstenacigenins A and B, along with a known compound, dresgenin, were isolated from the mild acid hydrolysate of the ethanol extract of the stems of *Marsdenia tenacissima*. Their structures were deduced by a combination of 1D and 2D NMR spectroscopic techniques as 12β-cinnamoyldihydrosarcostin and 12β,20-dibenzoyldihydrosarcostin, respectively.

INTRODUCTION

Marsdenia tenacissima (Roxb.) Wight et Arn. (Asclepiadaceae) is a medicinal herb distributed in the southwest region of the People's Republic of China. Its stem has been used as a folk remedy for the treatment of cancer and tracheitis for a long time in China. The isolation and structural elucidation of six polyoxypregnanes were demonstrated in our previous paper [1]. Further phytochemical studies resulted in the isolation of three minor polyoxypregnane esters, among which two are new compounds. This paper principally deals with the isolation and structural determination of marstenacigenin A (**2**) and B (**3**) and the NMR data assignment of the known compound dresgenin (**1**).

RESULTS AND DISCUSSION

Compound **1**, dresgenin (12β-benzoyldihydrosarcostin, amorphous powder, mp 141–142°), was first isolated from the rhizomes of the asclepiadaceous plant *Dregea sinensis* var. *corrugata* [2]. Its UV absorptions at 278 and 258 nm, IR absorptions at 1700, 1600, 1450 and 1280 cm^{-1}, as well as the EI mass spectral base peak fragment at *m/z* 105 (benzoyl cation) and strong ion at *m/z* 122 (benzoic acid), indicated the presence of a benzoyl group. The CI mass spectrometry of **1** gave an apparent quasimolecular ion $[M + 1]^+$ at *m/z* 471, and the EIHR mass spectrum at *m/z* 470.2675 corresponded to the molecular formula $C_{28}H_{38}O_6$ (calc. 470.2668). However, the ^{13}C NMR spectrum of **1** showed the presence of six oxygen-bearing carbons in the 70–90 ppm region; thus, the molecular formula of **1** should be $C_{28}H_{40}O_7$, recognizing the presence of a carbonyl group of the benzoyl ester also in the molecule. The apparent molecular ion at *m/z* 470 most likely arises from the M^+ 488 ion through a loss of H_2O. As shown previously [3], polyoxypregnanes rarely give a molecular ion in their EI mass spectra, and, in most cases, the significant fragment ions are those which arise from $[M]^+$ with the loss of one or several molecules of H_2O.

The attachment of a benzoyl group on dihydrosarcostin (deacyldrevogenin) [4, 5] in **1** was clarified by its ^1H NMR spectrum. The H-12 resonance was shifted to lower field at δ4.82 (*dd*, $J = 4.4$ and 11.2 Hz), and in contrast, the chemical shift of H-20 was observed at higher field at δ 3.66 (*q*, $J = 6.3$ Hz), indicating benzoyl group attachment to 12β-OH. The unambiguous assignment of the ^{13}C NMR spectrum of **1** (Table 1) was resolved by a combination of APT, DQF–COSY, HETCOR and long-range ^1H–^{13}C correlation spectra (selective INEPT and FLOCK, Fig. 1), in a similar way as with gymnemarsgenin [3].

It was also observed with some interest that, in the ^1H NMR spectrum of **1**, the signal of the 21-CH_3 resonated at δ1.02 ppm, lower field than the 19-CH_3, but higher field than the 18-CH_3, i.e. 18-CH_3 > 21-CH_3 > 19-CH_3. The sequence of the carbon chemical shifts of the three methyl groups in the ^{13}C NMR spectrum appears as 19-CH_3 > 21-CH_3 > 18-CH_3, the order of 18- and 19-CH_3 being reversed in comparison with the ^1H NMR spectrum. These results are consistent with those observed for gymnemarsgenin [3].

Compound **2** molecular formula $C_{30}H_{42}O_7$, the CI mass spectrum of which showed a very weak quasimolecular

*Part 6 in the series 'Studies on the C-21 steroids from Chinese Asclepiadaceae plants'. For part 5 see ref. [3].

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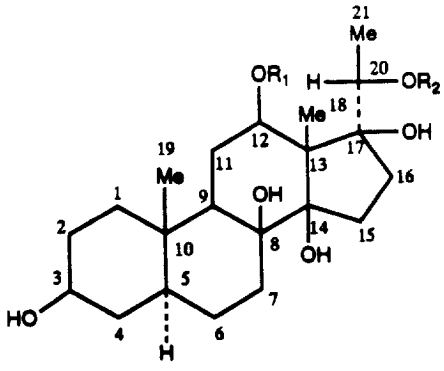

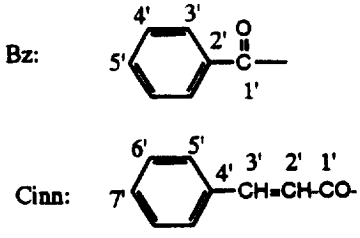

	R_1	R_2
1	Bz	H
2	Cinn	H
3	Bz	Bz

Table 1. ^{1}H and ^{13}C NMR data for compounds **1–3**
(δ values in ppm from internal standard TMS; H coupling in Hz)

	^{13}C NMR data			^{1}H NMR data		
	1	**2**	**3**	**1**	**2**	**3**
1	37.73	37.72	37.63			
2	30.73	30.77	30.68			
3	71.17	71.25	71.15	3.58 (*m*)	3.61 (*m*)	3.60 (*m*)
4	37.46	37.48	37.40			
5	45.32	45.36	45.23			
6	24.37	24.39	24.33			
7	34.04	34.10	33.95			
8	75.51	75.49	75.71	8-OH: 2.55	8-OH: 2.51	8-OH: 2.48
9	46.32	46.41	46.02			
10	36.01	36.07	35.99			
11	23.66	23.63	24.02			
12	75.31	75.49	74.10	4.82 (*dd*, 4.4, 11.2)	4.71 (*dd*, 4.0, 11.0)	4.89 (*dd*, 5.0, 11.5)
13	56.54	56.35	56.68			
14	87.93	87.94	87.96	14-OH: 4.75	14-OH: 4.66	14-OH: 4.61
15	32.97	32.97	32.73			
16	31.52	31.53	31.87			
17	87.90	87.94	87.72	17-OH: 3.36	17-OH: 3.13	17-OH: 3.04
18	11.74	11.82	11.03	1.60	1.54	1.59
19	17.96	17.44	14.88	0.95	0.99	0.97
20	70.93	71.20	74.29	3.66 (*q*, 6.3)	3.66 (*q*, 6.0)	4.60 (*q*, 6.0)
21	12.52	12.53	12.48	1.02 (*d*, 6.3)	1.09 (*d*, 6.0)	1.16 (*d*, 6.0)
	Bz	Cinn	Bz			
1'	165.77	166.11	168.56			
2'	130.10	117.3	131.03	6.44 (*d*, 16.5)		
3'	129.56	146.19	129.39	8.07 (*dd*, 8.0, 1.5)	7.75 (*d*, 16.5)	7.95 (*dd*, 8.0,1.5)
4'	128.72	133.94	128.17	7.47 (*t*, 8.0)		7.44 (*dd*, 8.0, 1.5)
5'	133.47	128.93	132.98	7.59 (*t*, 8.0)	7.24 (*t*, 7.4)	7.56 (*dd*, 8.0, 1.5)
6'		128.30			7.55 (*dd*, 7.4, 1.5)	
7'		130.70			7.40 (*t*, 7.4)	

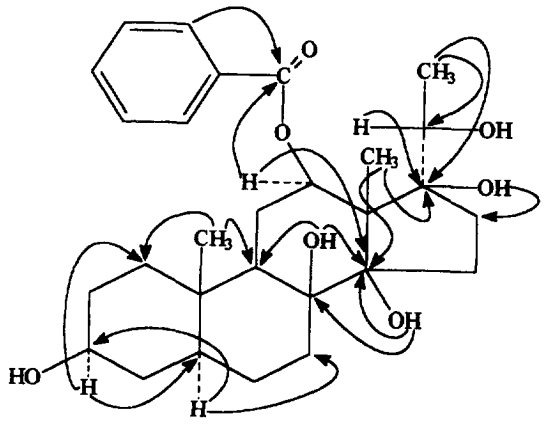

Fig. 1. ^{1}H–^{13}C NMR long-range correlations of dresgenin (**1**) by FLOCK and selective INEPT.

ion $[M + 1]^+$ at *m/z* 515. Similarly to **1**, the most significant ion of **2** in the EI mass spectrum is $[M^+ - H_2O]$ at *m/z* 496, EIHR mass spectrum *m/z* 496.2826, corresponding to a molecular formula $C_{30}H_{40}O_6$ (calc. 496.2824). Other fragment ions in the EI mass spectrum included *m/z* 478 $[M^+ - 2H_2O]$, 366 $[M^+ -$ cinnamic acid], 348 $[366 - H_2O]$ and a base peak at *m/z* 131 (cinnamoyl cation). The very similar IR, UV and NMR spectroscopic appearance of **2** with respect to **1** suggested its analogy with **1**. The relative bathochromic absorption of the carbonyl group (1640 cm^{-1}) in the IR spectrum, combined with the base peak of the EI mass spectrum at *m/z* 131 (cinnamoyl cation) suggested that there is a cinnamoyl group at the 12β-OH of **2** instead of the benzoyl group of **1**. Therefore, the structure of **2** was deduced as 12β-cinnamoyldihydrosarcostin. The ^{13}C NMR chemical shifts of **2** could be attributed with reference to **1**.

Compound **3**, molecular formula $C_{35}H_{44}O_8$ (M_r 592) based on its ^{13}C NMR spectrum, showed an apparent molecular ion $[M^+ - H_2O - 2H]$ at *m/z* 572 in the EI mass spectrum, and other predominant fragment ions at *m/z* 556 $[M^+ - 2H_2O]$, 538 $[M^+ - 3H_2O]$, and 105 (benzoyl cation). The IR and UV spectra of **3** were identical with those of **1**, moreover, the ^{1}H NMR of **3** is nearly the same as that of **1** with the exception that the signal of 20-H was also shifted to low field at δ4.61 (*q*, $J = 6.0$ Hz), in addition to the 12α-H resonating at δ 4.89 ($J = 5.0$ and 11.5 Hz). Based on spectral evidence, **3** was identified as 12β,20-dibenzoyldihydrosarcostin.

The NMR features of the acyl groups of **3** were of interest because the aromatic carbons and protons of the two benzoyl groups resonated coincidentally, but there were two carbonyl group signals observed in its ^{13}C NMR spectrum. In accordance with the results of selective INEPT and FLOCK experiments, the carbonyl group attached to the 12β-OH resonated at δ166.03 ppm, whereas the carbonyl group attached at 20-OH resonated at δ168.56 ppm, since the long-range ^{1}H–^{13}C NMR correlations were detected between 12α-H and δ166.03 ppm, as well as 20-H and δ168.56 ppm. This suggests that the carbonyl groups are located in different chemical and spatial environments, but that the anisotropic effects on the benzoyl rings are not noticeable.

The lower field shift of 2.53 ppm in the ^{13}C NMR spectrum of the C-20 benzoyl group than that at the C-12 position implies that the latter is more hindered than the former, which could be clarified by a molecular modelling study and suggests and explanation for the observed reactivity difference of acylation between the C-12-OH and C-20-OH of sarcostin [6], as well as the diesters of utendin and tomentogenin on mild hydrolysis [7].

EXPERIMENTAL

General. Mps: uncorr. UV spectra were recorded in MeOH soln on a Beckman DU-7 spectrometer. IR spectra were recorded in a KBr pellet on a MIDAC FT-IR interferometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The NMR, COSY, HETCOR and ROESY spectra were recorded at 500.12 MHz for ^{1}H and 125.76 MHz for ^{13}C with a GE OMEGA 500 instrument, using GE standard programs in $CDCl_3$ soln. APT, FLOCK and selective INEPT experiments were recorded at 90.8 MHz with a Nicolet NMC-360 instrument; $^{n}J = 6.3$ Hz was used in the FLOCK experiment. For the selective INEPT experiments, data sets of 16 K covering a spectral width of 10 kHz were acquired. Proton pulse widths were calibrated using a sample of HOAc in 10% C_6D_6 ($^{1n}J = 6.7$ Hz) in a 5-mm NMR tube [8]. CI (CH_4 reagent) and EI mass spectra (70 eV) were recorded with a Varian MAT-112S mass spectrometer, and high resolution mass spectra were recorded with a Finnigan MAT-90 instrument.

Plant material. The stems of *M. tenacissima* were collected in October 1988, from Yunnan Province, People's Republic of China, and identified by Dr Mi Jiang. A voucher specimen is deposited in the herbarium of Shanghai Institute of Pharmaceutical Industry, Shanghai, China.

Extraction and separation. The concd EtOH extract from the stem (10 kg) of the title plant was extracted with $CHCl_3$, and the extract concd and then partitioned with petrol. The deposited glycosides were collected and hydrolysed by refluxing with an equal vol. of MeOH and 0.1 N H_2SO_4 soln for 30 min. The reactive liquid, after removal of MeOH *in vacuo*, was extracted with Et_2O to afford an extract containing the polyoxypregnane ester aglycones, which was subjected to silica gel CC, eluting with $CHCl_3$ and an increasing percent of MeOH. Final sepn of compounds **1** (15 mg), **2** (18 mg) and **3** (11 mg) was achieved by prep. HPLC [Waters L 244, column: 9 × 250 mm, 10 μm, ODS C_{18} 10 × m; solvent: MeOH–H_2O (7:3); monitored by refractometer].

Marstenacigenin A (**2**). Amorphous powder (18 mg, 0.00018%), mp 135–7°, $[\alpha]_D + 47.6°$ (*c* 0.6, MeOH), UV λ_{max}^{EtOH} nm (log ε): 217 (4.14), 222 (4.09), 227 (4.26). IR ν_{max} (cm^{-1}): 3470, 1698, 1635, 1595, 1575, 1450, 1280. EIMS *m/z* (rel. int.): 496 $[M^+ - H_2O$, 12], 478 $[M^+ - 2H_2O$, 24], 366 $[M^+ -$ cinnamic acid, 45], 348

[366 − H_2O, 34], 330 [348 − H_2O, 28], 148 (cinnamic acid, 35), 131 (cinnamoyl cation, 100). ^1H and ^{13}C NMR data: see Table 1.

Marstenacigenin B (**3**). Amorphous powder (11 mg, 0.00011%), mp 110–112°, $[\alpha]_D$ + 20.0° (*c* 0.7, MeOH), UV λ_{max}^{EtOH} nm (log ε): 278 (3.2), 257 (3.30), 229 (4.05). IR ν_{max} (cm^{-1}): 3420, 1700, 1600, 1450, 1280. EIMS *m/z* (rel. int.): 572 [M^+ − H_2O − 2H, 18], 556 [M^+ − $2H_2O$, 9], 538 [M^+ − $3H_2O$, 8], 470 [M^+ − benzoic acid, 35], 452 [M^+ − benzoic acid − H_2O, 28], 330 [M^+ − 2× benzoic acid, 20], 105 (benzoyl cation, 100). ^1H and ^{13}C NMR data: see Table 1.

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