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STRUCTURE REVISION AND INTERNAL ACYL MIGRATION OF GYMNEMARSGENIN

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Key Word Index—*Gymnema yunnanense*; *Marsdenia globifera*; pregnane steroid; NMR assignments; structure revision; gymnemarsgenin.

Abstract—Reinvestigation of the NMR spectra of gymnemarsgenin by the selective INEPT and various two-dimensional NMR techniques (DQF-COSY, ROESY, HETCOR) led to the structural revision of gymnemarsgenin as 12β-*O*-cinnamoyl-20-*O*-benzoyl sarcostin. The ^{1}H and ^{13}C NMR spectra were completely assigned. It is apparent that the 12-cinnamoyl group migrated to C-20 under the mild alkaline conditions rendered for partial saponification.

INTRODUCTION

Gymnemarsgenin (**1**) is a C-21 steroid isolated by us from the Chinese medicinal asclepiadaceae plants *Gymnema yunnanense* Tsiang [1] and *Marsdenia globifera* Tsiang [2]. It was deduced to be a cinnamoyl and benzoyl diester of sarcostin (**2**), a polyoxypregnane steroid. A monoester product, 20-*O*-cinnamoylsarcostin (**3**) was obtained from the partial saponification of **1** under mild alkaline conditions (K_2CO_3–methanol at 25° for 5 hr), and thus the structure of **1** was deduced to be 12β-*O*-benzoyl-20-cinnamoylsarcostin [1]. However, by direct analysis of **1** using a combination of NMR techniques, especially the selective INEPT technique it has been unexpectedly found now that the benzoyl is linked to the hydroxyl group at C-20 and the cinnamoyl to the hydroxyl at C-12; a result opposite to that inferred from partial saponification [1]. Therefore, the initially published structure of gymnemarsgenin should be revised. It is evident that the C-12 cinnamoyl group migrated to C-20 under the mild saponification conditions. A possible mechanism for the acyl migration is discussed.

RESULTS AND DISCUSSION

Gymnemarsgenin (**1**) and other four known steriods, sarcostin (**2**), penupogenin (**4**), metaplexigenin and 20-*O*-cinnamoylsarcostin (**3**), were isolated from the acidic hydrolysate of the crude glycosides of *G. yunnanense* [1]. Compound **1** was also isolated as the major steroid from *M. globifera*, along with the known compounds **2**, penupogenin, **3**, isolineolon, gagaminin (**5**), and two new compounds, 20-*O*-benzoylsarcostin (**6**) and 12-*O*-benzoylsarcostin (**7**) [2].

Compound **1**, $C_{37}H_{44}O_8$, has the following physical properties: mp 155–7°, $[\alpha]_D$ + 145.9° ($CHCl_3$, *c* 0.51); UV λ_{max} (log ε, EtOH): 218 (4.25), 224 (4.29), 232 (4.14), 280 (4.16) nm; IR ν_{max} (cm^{-1}) 3450, 1708, 1640, 1600, 1575, 1490, 1450, 1280. Its mass spectrum showed no parent peak at *m/z* 616, but other peaks suggested fragmentation from this molecular ion: *m/z* 580 ($M^+ - 2H_2O$), 468 (M^+ – cinnamic acid), 450 (468 – H_2O), 432 (450 – H_2O), 346 (M^+ – cinnamic acid – benzoic acid), 328 (346 – H_2O), 310 (328 – H_2O), 295 (310 – Me), 131 (cinnamoyl cation), 105 (benzoyl cation). Compound **2** was released from **1** by saponification with 5% methanolic potassium hydroxide. The ^{1}H NMR spectrum of **1** showed the chemical shift of H-12 at δ5.28 (*dd*, *J* = 11.4 Hz) and of H-20 at δ5.25 (*q*, *J* = 6 Hz), i.e. at substantially lower field than the corresponding resonances of **2**, δ3.93 and δ4.46, respectively. The spectral and chemical evidence cited above suggested that **1** was a diester of **2** with acylation by cinnamic acid and benzoic acid moieties.

HETCOR and selective INEPT experiments were carried out in order to determine unambiguously the precise locations of the acyl (benzoyl and cinnamoyl) substituents in this sarcostin diester. Selective INEPT spectra were obtained by irradiating the respective protons to display the long-range chemical shift correlations between ^{13}C and ^{1}H three bonds apart. Selective irradiation of H-12 (δ5.28, *dd*) enhanced the carbon signal at δ167.2; irradiation of the α-olefinic proton of the cinnamoyl group at δ7.81 also enhanced the carbon signal at δ167.2, thus clearly demonstrating that the cinnamoyl

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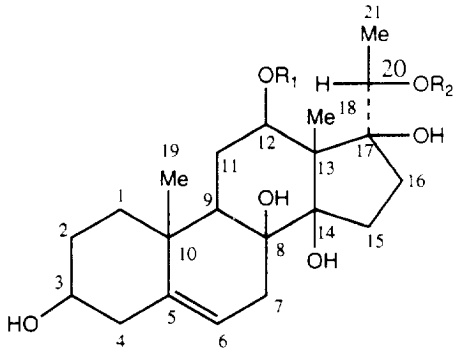
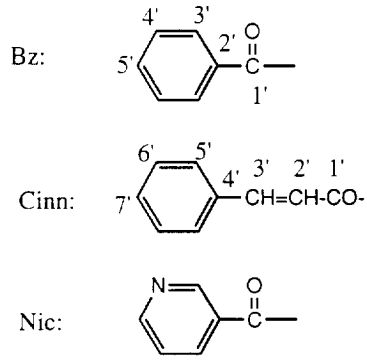




1. R_1 = Cinn, R_2 = Bz

2. $R_1 = R_2$ = H

3. R_1 = H, R_2 = Cinn

4. R_1 = Cinn, R_2 = H

5. R_1 = Cinn, R_2 = Nic

6. R_1 = H, R_2 = Bz

7. R_1 = Bz, R_2 = H

group was attached to the hydroxyl group at C-12. Selective irradiation of H-20 at δ5.25 (correlated with the carbon shift of δ76.3 in the HETCOR spectrum, and coupled to the methyl group at δ1.57 by COSY) resulted in enhancement of the carbon resonances at δ166.0 (benzoyl carbonyl carbon), 57.5 (C-13), 88.0 (C-17, 2-bond coupling) and 34.2 (C-16), permitting the assignment of the benzoyl group to the hydroxyl moiety at C-20.

The results described above unequivocally demonstrated that the structure of **1** was 12β-*O*-cinnamoyl-20-*O*-benzoylsarcostin. Based on the information from DQF-COSY, ROESY and selective INEPT (Table 1), a computer-assisted three-dimensional structure (Fig. 1) was obtained by the molecular modelling program PCMODEL 386 V 4.0, using MMX force field calculations for energy minimization. The calculated distances between H_3-19 and H-1β, H_3-19 and H-4β were 2.34 and 2.17 Å, respectively, consistent with the strong ROESY correlation between each of these pairs, and thus the 19-CH_3 can be selected as the starting point for the ^{1}H and ^{13}C NMR assignments (Table 2).

In most of the previous literature, the ^{13}C NMR chemical shift of the C-18 was assigned at lower field than that of C-19, but our selective INEPT results clearly indicated that the shift order of C-18 and C-19 should be revised, because irradiation of the methyl proton resonance at δ1.32 (correlated to carbon shift at δ18.6 by HETCOR), resulted in the enhancement of the olefinic carbon at δ140.6 (C-5); irradiation of the methyl proton at δ2.12 (shown to be connected to the carbon resonance at δ12.0 by HETCOR) resulted in the enhancement of carbon signals at δ89.4 (C-14) and 88.0 (C-17) so that δ18.6 should be the chemical shift of C-19, and δ12.0 the shift of C-18. Our results also indicated that C-15 (δ34.5) was shifted to lower field than that of C-16 (δ34.2), and these unequivocal assignments are reported here for the first time.

As indicated in our previous report [1], **1** produced **2** and **3** under mild saponification conditions. It is apparent that the saponification product **3** was formed by loss of the acyl group attached to the hydroxyl at C-20, followed by acyl migration from the hydroxyl group of C-12 to C-20 during the hydrolysis. Hayashi and Mitsuhashi [3] have also reported the acyl migration from C-12 and C-20 in **5** and dehydrotomentosin [4] under mild alkaline hydrolysis conditions. The acyl migration between C-12 and C-20 of wilforine [5], and between C-12 and C-11 of the cundurango glycosides [6] were also reported. However, the reference value of the latter two citations was limited due to the lack of substantial structure determination of either substrate or product. Hayashi and Mitsuhashi [3] reported that **5** formed

Table 1. Summary of major results from the COSY, ROESY and selective INEPT spectra of gymnemarsgenin (**1**)*

Proton	COSY (proton)	ROESY (proton)	Selective INEPT (carbon)
1α	1β,2a,2β	1β,2β	
1β	1α,2α,2β	1α,2α,2β,19-H	
2α	1α,1β,2β	1β,2β	
2β	1α,1β,2α	1α,1β,2α	
3α	2α,2β,4α,4β	2α,2β,4β	
4α	3α,4β	4β	(5), 10
4β	3α,4α	3α,4α	(5), 10
6	7β	7β	(5), 8, 4
7α	7β	7β	
7β	6,7α	6,7α	
9α	11α,11β	11α	
11α	9α,11β,12α	11β,12α	
11β	9α,11α,12α	11α	
12α	11α,11β	11α	–CO (Cinn), (13), 14, 17, 18
15α	15β,16α,16β	15β,16α,16β	
15β	15α,16α,16β	15α,16β,16β	
16α	15α,15β,16β	15α,15β,16β	
16β	15α,15β,16α	15α,15β,16α	
18		11β,15β,16β	14, 17, 12, (13),
19		1β,4β	5,9,1,(10),
20	21-H	21-H	–CO (Bz), 13, (21), 16
21	20-H	20-H	17, (20),
Bz			
3′			CO (Bz), (4′)
Cinn			
2′			–CO (Cinn), 4′
3′			CO (Cinn), 5′

*Two-bond couplings between ^{1}H and ^{13}C are given in parentheses.

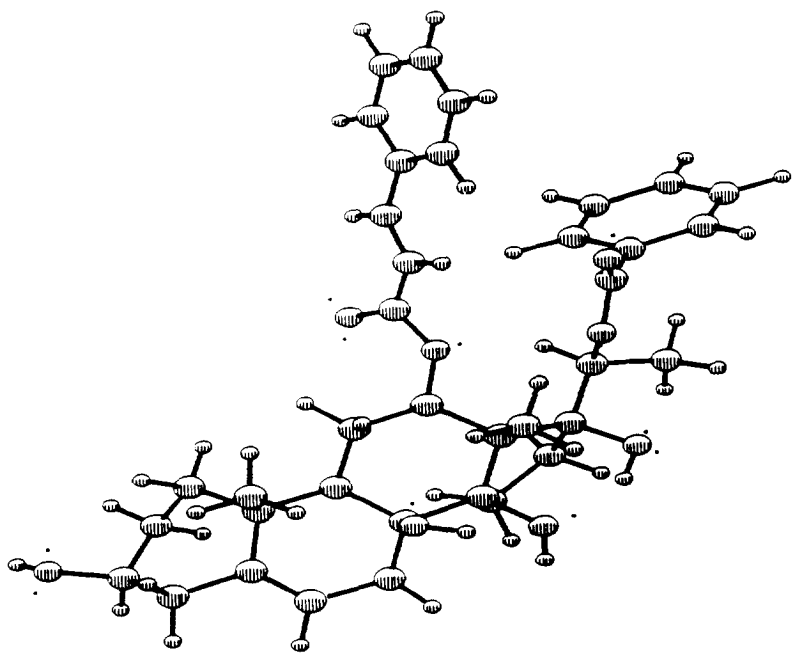


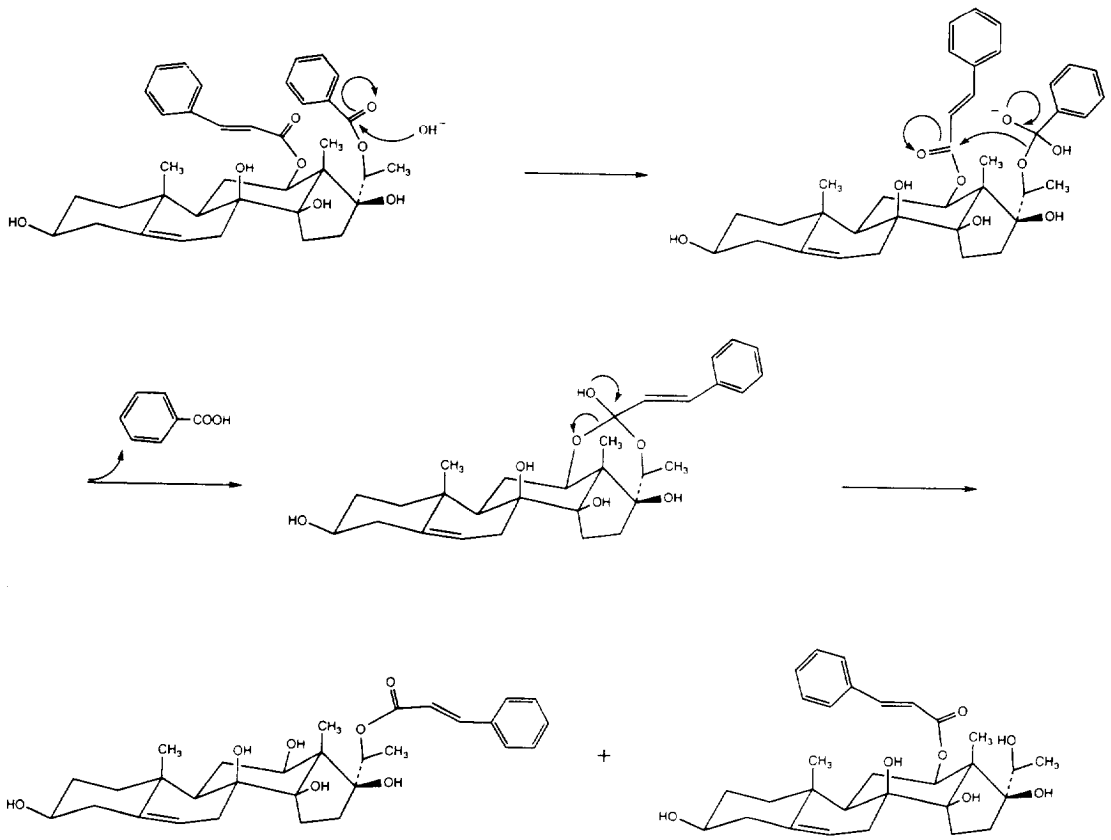
Fig. 1. A steric view of gymnemarsgenin (**1**) by computer modelling.

Table 2. ^1H and ^{13}C NMR data for gymnemarsgenin (**1**)†

	^{13}C	^1H		
1	39.4	α: 1.07 (*dt*, 12.0, 3.5)		β: 1.68 (*m*)
2	32.4	α: 1.94 (*m*)		β: 1.82 (*dt*, *J* = 12.0, 3.5)
3	71.9	α: 3.81 (*m*)		
4	43.7		2.56 (*m*)	
5	140.6			
6	119.1		5.32 (*br d*, 4.5)	
7	35.3	α: 2.33 (*d*, 12)		β: 2.43 (*dd*, 4.5, 12)
8	74.8			
9	44.5	α: 1.74 (*d*, 2.5)		
10	37.7			
11	26.1	α: 2.04 (*m*)		β: 2.35 (*m*)
12	75.1	α: 5.28 (*dd*, 11,4)		
13	57.5			
14	89.4			
15	34.5	α: 2.00 (*m*)		β: 2.09 (*m*)
16	34.2	α: 2.03 (*m*)		β: 2.12 (*m*)
17	88.0			
18	12.0		2.12 (*s*)	
19	18.6		1.32 (*s*)	
20	76.3		5.25 (*q*, 6)	
21	15.8		1.50 (*d*, 6)	
Bz				
1′	166.0			
2′	131.6			
3′	130.6		8.17 (*d*, 7.6)	
4′	130.8		7.32 (*m*)	
5′	133.8		7.48 (*t*, 7.6)	
Cinn				
1′	167.2			
2′	120.7		6.45 (*d*, 16)	
3′	144.3		7.81 (*d*, 16)	
4′	135.3			
5′	129.0		7.28 (*m*)	
6′	128.9		7.36 (*m*)	
7′	129.5		7.31 (*m*)	

*ppm from internal standard TMS in pyridine-d_5.
†Multiplicity and coupling constants in parentheses.

3 (migration product) and **7** (C-12-OH acyl intention product) in K_2CO_3 solution in methanol, and only the migration product was detected with dehydrotomentosin under the same conditions. However, we only obtained the migration product **3** from **1** under the same conditions, and the C-12-*O*-acyl retention product was not detected. Although there is a little difference between the migration results of the two research groups (probably because the reaction conditions were not completely consistent), it is apparently a phenomenon that C-20-*O*-acyl retention products cannot be obtained under these conditions. In Scheme 1 a tentative mechanism is proposed to explain the above experimental observations. In a polar solvent system such as methanol, there is a strong hydrophobic effect to force the two aromatic moieties of benzoyl and cinnamoyl to approach each other. In the case of C-12, C-20 di-*O*-acyl ester-OH and C-20-OH diester, following attack of the carbonyl carbon by the hydroxyl anion, molecular modelling (Fig. 1) studies showed that the cinnamoyl group lies in a sterically hindered environment. Hydroxyl anion will be most likely to attack the less hindered carbonyl carbon of the benzoyl group at C-20-OH rather than the C-12-*O*-cinnamoyl group. Benzoate is then lost followed by formation of a seven-membered ring *ortho*-ester anion intermediate. Further decomposition of this intermediate by ring opening provides the migration product **3** and the C-12-*O*-acyl retention product. The formation of a migration product is more thermodynamically favourable because of the steric constraints of the C-21-*O*-acyl group (steric assistance) so that the migration product is the major product, and the C-12-*O*-acyl retention products were either not detected (as with **1**) or were minor (as with **5** [3]). In conclusion, mild saponification may lead to an erroneous structure elucidation for the placement of esterifying functionalities and is unsuitable for the structural determination of diesters of **2** and possibly for other pregnane steroid diesters.



Scheme. 1. A tentative mechanism for the internal acyl migration of gymnemarsgenin (1).

EXPERIMENTAL

Mps (uncorr.) were determined on a Kofler hot stage apparatus. Optical rotation was measured with a JASCO-20C apparatus at room temp. IR spectra were recorded on a Perkin-Elmer 577 spectrometer. Mass spectra were determined on JEOL JMS-OISF-2 and Finnigan-4510 spectrometers. ^{1}H, ^{13}C, DEPT, COSY, DDQF-COSY, ROESY and HETCOR spectra were taken on a GE OMEGA 500 instrument operating at 500.1 MHz for ^{1}H and homonuclear 2D NMR spectra, 125.8 MHz for ^{13}C spectra, and 500.1/125.8 MHz for HETCOR spectrum using standard GE programs in pyridine-d_5 soln, and have been described in detail previously [7, 8]. Selective INEPT spectra were recorded on a Nicolet NT-360 instrument operating at 90.8 MHz, with $J = 6$ Hz for aliphatic protons.

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