Pregnanes and Pregnane Glycosides from *Hoya carnosa*

Fumiko ABE,[a] Hitomi FUJISHIMA,[a] Yukiko IWASE,[a] Tatsuo YAMAUCHI,*[,a] Kazuhiko KINJO,[b] and Shiryo YAGA[b]

Faculty of Pharmaceutical Sciences, Fukuoka University,[a] 8–19–1 Nanakuma, Jonan-ku, Fukuoka 814–0180, Japan, Faculty of Agriculture, University of the Ryukyu,[b] Nishihara-cho, Nakagami-gun, Okinawa 903–0113, Japan.
Received March 19, 1999; accepted May 10, 1999

Eleven pregnanes were isolated from the hydrolysate of the $CHCl_3$ extract fractionated from the caules of *Hoya carnosa*. Among these, six pregnanes, including 19-acetoxydigipurpurogenin II, were new, and their structures were elucidated. The structures of twenty new pregnane tetraosides and pentaosides, named hoyacarnosides A—T, besides three known ones from the $CHCl_3$ extract, were determined.

Key words *Hoya carnosa*; Asclepiadaceae; pregnane glycoside; sakuragenin; hoyacarnoside

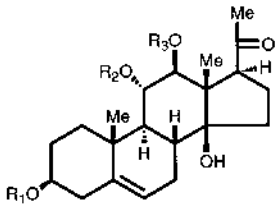
Pregnanes in Asclepiadaceae plants have been studied actively since the 1960s, and many pregnanes and pregnane glycosides were disclosed in several genera. *Hoya carnosa* R. BR. is one of the liana belonging to Asclepiadaceae and grown in the Ryukyu district. The pregnane ingredients in this plant, however, have not yet been investigated, although cyclitols were studied.[1)] As a part of our investigation of the steroidal constituents from Apocynaceae[2)] and Asclepiadaceae[3)] plants, pregnanes and pregnane glycosides from the caules of this plant were examined in advance of those from the leaves.

When the air-dried caules of *H. carnosa* were percolated with MeOH and the MeOH extract was partitioned with $CHCl_3$–H_2O, pregnan glycosides were observed in the $CHCl_3$ fraction. After a portion of the $CHCl_3$ extract was hydrolyzed under mild conditions, pregnanes and sugars were isolated using a silica gel column. From the pregnane fraction, eleven pregnanes (a-**1**—a-**11**) were obtained.

Five known pregnanes were identified to be drevogenin P (a-**1**),[4)] drevogenin A (a-**3**),[4)] 17β-marsdenin (a-**4**),[5)] drebysso-genin J (a-**8**)[6)] and marsectohexol (a-**9**),[7)] based on comparisons of the NMR and MS data with those in the literature.

The high resolution (HR)-FAB-MS of a-**2** suggested the molecular formula $C_{25}H_{36}O_7$, which was two acetyl residues greater than a-**1**. The ^{13}C-NMR signals showed a similar pattern to those of a-**1** and a-**3**. The presence of acetyl groups was confirmed by two 3H-singlet signals in the ^{1}H-NMR spectrum (δ 1.96, 2.10). In a-**2**, two carbinyl protons observed at δ 3.67 (t, J=10 Hz) and δ 3.05 (d, J=10 Hz) in a-**1** were shifted to δ 5.36 (t, J=10 Hz) and δ 4.83 (d, J=10 Hz), respectively, with almost the same chemical shifts as those in 11,12-diacylated pregnanes such as a-**3**. The structure of a-**2** was thus assigned to be 11,12-di-*O*-acetyldrevogenin P.

The relation similar to that between a-**2** and a-**1** was seen in comparison of the ^{1}H-NMR spectra between a-**5** and a-**4**. The presence of two acetyl groups was suggested in a-**5**, based on two 3H-singlet signals at δ 1.99 and 2.09, and also on the lower field shifts of two carbinyl protons in comparison with those of a-**4** ($\Delta\delta$=1.62, 1.74 ppm, respectively). During the NMR measurement, the H-21 signal became am-



a-**1** : R_1=R_2=R_3=H

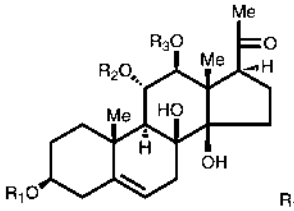
1 : R_1=S_1
2 : R_1=S_2
3 : R_1=S_3

a-**2** : R_1=H, R_2=R_3=Ac

4 : R_1=S_1
5 : R_1=S_3

a-**3** : R_1=H, R_2=Ac, R_3=Isoval

6 : R_1=S_1
7 : R_1=S_3



a-**4** : R_1=R_2=R_3=H

8 : R_1=S_1
9 : R_1=S_2
10 : R_1=S_3
11 : R_1=S_4

a-**5** : R_1=H, R_2=R_3=Ac

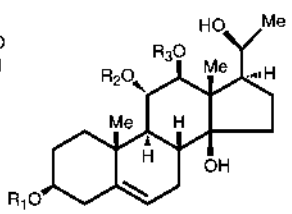
12 : R_1=S_1
13 : R_1=S_2
14 : R_1=S_3

a-**6** : R_1=H, R_2=Ac, R_3=Isoval

15 : R_1=S_1
16 : R_1=S_3

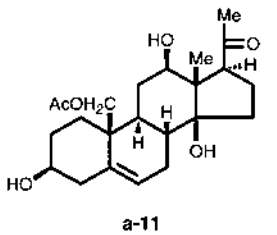
a-**7** : R_1=H, R_2=Ac, R_3=Bz

17 : R_1=S_1
18 : R_1=S_3

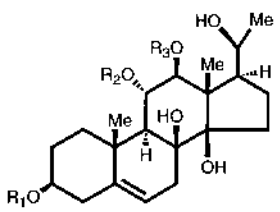


a-**8** : R_1=H, R_2=R_3=Ac

19 : R_1=S_1
20 : R_1=S_3



a-**11**



a-**9** : R_1=R_2=R_3=H

21 : R_1=S_3

a-**10** : R_1=H, R_2=R_3=Ac

22 : R_1=S_1
23 : R_1=S_3

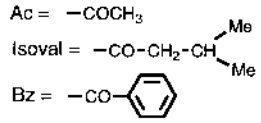


Chart 1

* To whom correspondence should be addressed.

Table 1. ^{1}H Spectral Data of a-**1**—a-**11** (δ ppm in CD_3OD)

H	a-**1**[a)]	a-**2**	a-**3**	a-**4**	a-**5**	a-**6**	a-**7**	a-**8**	a-**9**	a-**10**	a-**11**
3	3.44 (m)	3.39 (m)	3.38 (m)	3.47 (m)	3.42 (m)	3.43 (m)	3.44 (m)	3.43 (m)	3.42 (m)	3.47 (m)	3.48 (m)
6	5.44 (m)	5.48 (m)	5.48 (m)	5.33 (m)	5.32 (m)	5.32 (m)	5.35 (m)	5.48 (m)	5.30 (m)	5.36 (m)	5.66 (m)
9	1.27 (dd, 10, 12)	1.67 (t, 10)	1.68 (t, 10)	1.48 (d, 11)	1.89 (d, 11)	1.90 (d, 11)	1.97 (d, 11)	1.67 (t, 10)	1.49 (d, 11)	1.90 (d, 11)	1.25 (td, 13,4)
11	3.67 (t, 10)	5.36 (t, 10)	5.36 (t, 10)	4.07 (dd, 11, 10)	5.69 (t, 11)	5.69 (t, 11)	5.89 (dd, 11, 10)	5.39 (t, 10)	4.04 (dd, 11,10)	5.73 (dd, 11, 10)	1.54, 1.78 (m)
12	3.05 (d, 10)	4.83 (d, 10)	4.86 (d, 10)	3.17 (d, 10)	4.91 (d, 11)	4.94 (d, 11)	5.21 (d, 10)	4.80 (d, 10)	3.12 (d, 10)	4.87 (d, 10)	3.30 (dd, 12, 4)
17	3.61 (dd, 9, 5)	2.92 (dd, 9, 4)	2.91 (dd, 9, 5)	3.55 (dd, 9,4)	2.90 (dd, 9, 5)	2.89 (t, 7)	3.02 (t, 7)	1.68 (ddd, 9, 5, 1)		1.64 (dd, 10, 5)	3.59 (dd, 8, 6)
18	0.94 (s)	1.05 (s)	1.05 (s)	1.11 (s)	1.23 (s)	1.23 (s)	1.36 (s)	1.14 (s)	1.38 (s)	1.33 (s)	0.93 (s)
19	1.18 (s)	1.13 (s)	1.13 (s)	1.39 (s)	1.19 (s)	1.19 (s)	1.22 (s)	1.16 (s)	1.19 (s)	1.23 (s)	3.94 (d, 12) 4.56 (d, 12)
20								3.88 (qd, 6, 1)	3.93 (qd, 6, 1)	3.90 (br q, 6)	
21	2.28 (s)	2.20 (s)	2.19 (s)	2.28 (s)	2.20 (s)	2.17 (s)	2.09 (s)	1.09 (d, 6)	1.11 (d, 6)	1.09 (d, 6)	2.27 (s)
11-Ac		1.96 (s)	1.95 (s)		1.99 (s)	1.98 (s)	1.67 (s)	1.98 (s)		2.01 (s)	19-Ac 2.05 (s)
12-Acyl		Ac 2.10 (s)	Isoval 2.20, 2.31 (dd, 15, 7) 2.11 (m) 1.01 (6H) (d, 6)		Ac 2.09 (s)	Isoval 2.18, 2.30 (dd, 15, 7) 2.10 (m) 1.61 (6H) (d, 6)	Bz 8.06 (2H) (br d, 8) 7.51 (2H) (br t, 8) 7.66 (br t, 8)	Ac 2.04 (s)		Ac 2.05 (s)	

a) Dissolved in CD_3OD–$CDCl_3$.

Table 2. ^{13}C Spectral Data of a-**1**—a-**11** (δ ppm in CD_3OD)

C	a-**1**[a)]	a-**2**	a-**3**	a-**4**	a-**5**	a-**6**	a-**7**	a-**8**	a-**9**	a-**10**	a-**11**
1	39.6	39.6	39.6	40.9	41.2	41.2	41.2	39.4	41.6	40.7	34.5
2	31.7	32.3	32.3	31.4	31.9	31.9	31.9	31.8	32.0[b)]	31.3	32.0
3	71.8	71.9	71.9	72.3	72.6	72.5	72.5	71.2	71.4	71.8	71.5
4	42.8	43.3	43.3	42.8	43.3	43.3	43.3	42.7	43.4	42.7	42.5
5	140.8	141.0	141.0	141.4	140.9	140.9	141.0	140.1	142.2	140.5	135.4
6	121.9	122.8	122.8	118.1	118.8	118.8	118.8	122.3	118.6	118.3	126.6
7	28.1	28.7	28.8	35.8	35.9	35.9	36.0	28.0	36.2	35.4	27.7
8	37.0	38.1	38.0	76.1	76.5	76.5	76.7	37.2	76.5	75.7	37.7
9	48.5	48.9	49.0	50.1	49.7	49.7	49.5	48.3	51.0	48.9	44.1
10	39.2	40.0	40.0	39.2	39.7	39.7	39.7	38.9	39.9	39.0	40.8
11	71.7	72.9	72.9	70.4	72.6	72.5	72.5	72.9	73.0	72.4	30.7
12	78.0	78.6	78.2	78.4	79.1	78.6	79.6	78.8	80.0	78.8	73.6
13	55.3	55.3	55.5	55.7	56.0	56.2	56.4	53.8	55.4	54.4	56.2
14	85.5	85.3	85.2	85.9	86.2	86.3	86.4	83.4	85.9	84.8	87.0
15	35.2	35.1	35.1	37.4	37.4	37.5	37.5	33.8	30.7[b)]	31.3	34.4
16	24.7	24.7	24.7	24.9	24.9	25.0	25.0	18.4	19.1	18.5	24.9
17	58.1	59.0	59.0	58.9	60.0	60.1	60.0	51.8	52.9	52.4	57.9
18	10.0	11.3	11.3	12.2	13.4	13.5	13.5	10.2	11.8	12.4	9.0
19	19.0	19.5	19.6	17.6	18.3	18.3	18.3	19.4	17.8	18.1	65.1
20	219.2	216.5	216.6	218.8	216.4	216.6	216.2	65.3	66.6	65.5	219.6
21	32.9	32.0	32.2	32.6	31.7	31.7	31.6	22.1	21.9	21.9	32.8
11-Ac		171.8	171.8		171.5	171.5	171.6	171.3		170.9	19-Ac
		21.5	21.8		21.6	21.8	21.5	21.7		21.8	172.2
											21.0
12-Acyl		Ac	Isoval		Ac	Isoval	Bz	Ac		Ac	
		172.5	174.4		172.6	174.5	168.0	172.1		172.1	
		20.8	44.2		20.8	44.2	130.6	21.0		20.9	
			26.5			26.5	131.0 (×2)				
			22.9 (×2)			22.9 (×2)	129.8 (×2)				
							134.8				

a) Dissolved in CD_3OD–$CDCl_3$. *b*) Signal assignment may be interchangeable.

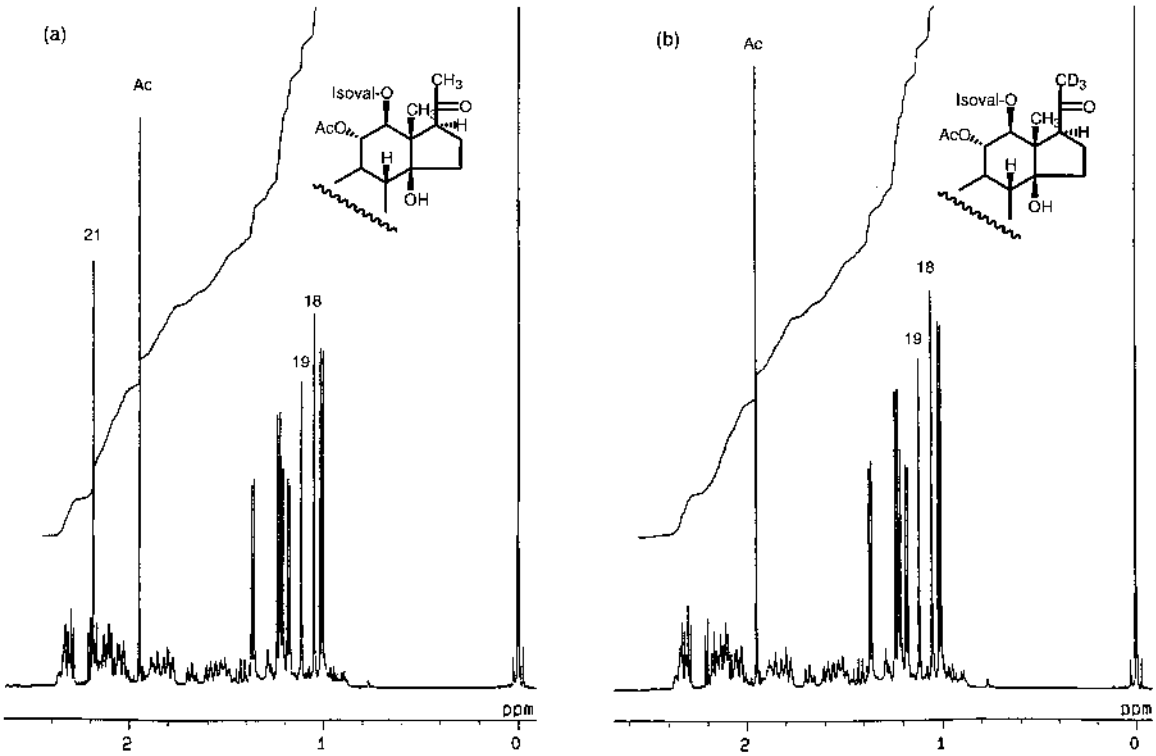


Fig. 1. ^{1}H-NMR Spectra of **6**, Deuterization of H-21 by CD_3OD
(a) One day after preparation of the solution. (b) One week after preparation of the solution.

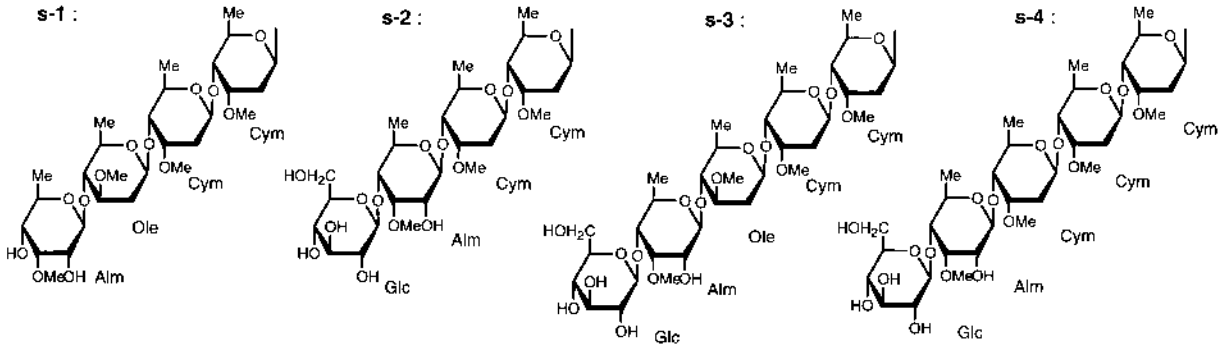
biguous in the CD_3OD solution, and finally disappeared after three weeks, accompanied by a broadened C-21 signal, as shown in the NMR chart of glycoside **6** (Fig. 1). The molecular formula of a-**5**, initially afforded by the $[M+Na]^+$ peak at *m/z* 487.2309, was consistent with 17β-marsdenin 11,12-diacetate. The recovered sample from the NMR measurement showed a $[M+Na]^+$ peak at *m/z* 490.2497, 3 mass units larger than a-**5**, suggesting the substitution of 3H at C-21 by 3D.

In the ^{1}H-NMR spectrum of a-**6**, acetyl (δ 1.98) and isovaleroyl residues (δ 1.61, 6H, d; δ 2.18, 2.30, 1H each, dd; δ 2.10, 1H, m) were observed, as in a-**3**. The location of acetyl and isovaleroyl residues at the 11- and 12-hydroxy groups were confirmed by the correlation of H-11β (δ 5.69) to the acetyl carbonyl carbon (δ 171.5), and of H-12α (δ 4.94) to the isovaleroyl carbonyl carbon (δ 174.5), respectively, in the heteronuclear multiple-bond correlation (HMBC) spectroscopy. Therefore, a-**6** was assigned to be 8-hydroxydrevogenin A (11-*O*-acetyl-12-*O*-isovaleroyl-17β-marsdenin). As observed in a-**5**, a-**6** also showed a $[M+Na]^+$ peak at *m/z* 532.2967, 3 mass units larger than the peak expected for the molecular weight, after recovering the sample from the NMR measurement in CD_3OD.

In the ^{1}H-NMR spectrum of a-**7**, the presence of acetyl and benzoyl groups was suggested by a 3H-singlet proton signal at δ 1.67, and by five proton signals due to a benzoyl residue at δ 7.51 (2H), 7.66 (1H), and 8.06 (2H). Most signals in the ^{1}H- and ^{13}C-NMR spectra coincided with those of a-**6**, except for signals due to a benzoyl residue instead of an isovaleroyl residue in a-**6**. Two carbinyl proton signals in the lower field assignable to H-11β (δ 5.89) and H-12α (δ 5.21) showed that the carbinols at C-11 and C-12 were acylated. Locations of the acyl groups were confirmed to be 11-*O*-acetyl and 12-*O*-benzoyl, based on the correlations between the 11β- and 12α-protons and two carbonyl carbons of acetic acid and benzoic acid, respectively, in the HMBC spectrum. In the HR-FAB-MS of a-**7**, a $[M+Na]^+$ peak was observed at *m/z* 552.2651 ($C_{30}H_{35}D_3O_8$+Na), as in a-**6**, after being dissolved in CD_3OD for two weeks. The structure of a-**7** was assigned to be 11-*O*-acetyl-12-*O*-benzoyl-17β-marsdenin (5,6-didehydrocynafogenin).

The molecular formula of a-**10** was suggested to be $C_{25}H_{38}O_8$ in the HR-FAB-MS peak at *m/z* 489.2465 ($[M+Na]^+$), and the acyl substituents were found to be two acetyl groups, since two 3H-proton signals were observed at δ 2.01 and 2.05, and H-11β and H-12α were observed in the lower field (δ 5.73, 4.87) in comparison with those of a-**9**. The presence of an 8β-hydroxy group was shown by a doublet signal of H-9 at δ 1.90 (J=11 Hz). The signals in the ^{13}C-NMR spectrum, including C-20 and C-16, which show different chemical shifts in C-20α and C-20β-carbinols,[8] were similar to those of a-**9**. The structure of a-**10** was thus assignable as 11,12-di-*O*-acetylmarsectohexol (12-*O*-acetyllanceogenin).

HR-FAB-MS of a-**11** afforded a $[M+Na]^+$ at *m/z* 429.2253, suggesting the molecular formula $C_{23}H_{34}O_6$. Only one tertiary methyl group assignable to C-18 or C-19 was shown in the ^{1}H- and ^{13}C-NMR spectra (δ 0.93, 9.0), besides one acetoxy residue (δ_H 2.05; δ_C 172.2, 21.0), a 17-acetyl group (δ_H 2.27; δ_C 219.6, 32.8) and the olefinic linkage at C-5/6. In the carbinyl proton region, two proton signals were observed at δ 3.48 (m) and 3.30 (dd, J=12.4 Hz), along with a couple of hydroxymethylene protons (δ 3.94, 4.56, each d, J=12 Hz). The former two proton signals were assigned to



Aglycone	s-1	s-2	s-3	s-4
a-1	1	2	3	
a-2	4		5	
a-3	6		7	
a-4	8	9	10	11
a-5	12	13	14	
a-6	15		16	
a-7	17		18	
a-8	19		20	
a-9			21	
a-10	22		23	

Chart 2

be H-3α and H-12α, the latter to be H-18 or H-19, based on their multiplicities. Four carbinol carbons were also observed in the ^{13}C-NMR spectrum: one each of the tertiary (δ 87.0) and the primary (δ 65.1) carbinol carbon signal, along with two secondary carbinol carbon signals (δ 71.5, 73.6) which were assignable to C-3 and C-12, resepctively, based on ^{13}C–^{1}H correlation spectroscopy (COSY) and distortionless enhancement by polarization transfer (DEPT). The nuclear Overhauser effect (NOE) correlation between H-12α and H-17 (δ 3.59) suggested the side chain at C-17 to be β. The hydroxymethylene protons at δ 3.94 and 4.56 showed correlation with the primary carbinol carbon signal at δ 65.1 in the ^{13}C–^{1}H COSY spectrum. As a result of the HMBC experiment, in which correlations were observed between the hydroxymethylene protons and the acetyl carbonyl carbon, C-5, C-9, C-10, the primary carbonyl carbon was assigned to be C-19. The methyl proton signal at δ 0.93 also showed correlations to C-12, C-14 and C-17, and the 18-methyl group was confirmed. The structure was thus determined to be 19-*O*-acetyl-3β,12β,14β,19-tetrahydroxypregn-5-en-20-one. Based on the Japanese name of this plant, "sakura-ran", a-**11** was named sakuragenin.

From the H_2O layer of the hydrolysate, four sugars, cymarose (Cym), oleandrose (Ole), 6-deoxy-3-*O*-methylallose (Alm) and glucose (Glc) were isolated. Based on their optical rotation values, four sugars were all identified to be in the D-series.

A $CHCl_3$ extract containing pregnane glycosides was then subjected to column chromatography (silica gel and octadecyl silica (ODS)) and HPLC to isolate twenty-three glycosides (**1**—**23**), including three known ones: dregeoside A_{p1} (**6**),[9] dregeoside A_{o1} (**7**)[9] and condurangoside D_{o1} (**10**).[5b] The sequence of the sugar moieties in the glycosides was examined using difference (DIF)-NOE measurement. Correlations of H-1 of sugar-1 to 3α-H of the aglycone, H-1 of sugar-2 to H-4 of sugar-1, H-1 of sugar-3 to H-4 of sugar-2, H-1 of sugar-4 to H-4 of sugar-3, and H-1 of sugar-5 to H-4 of sugar-4 were all observed in the DIF-NOE spectra.

All glycosides can be classified into four types of sugar sequences, ←Cym-Cym-Ole-Alm (type s-**1**), ←Cym-Cym-Alm-(4←1)-Glc (type s-**2**), ←Cym-Cym-Ole-Alm-(4←1)-Glc (type s-**3**), and ←Cym-Cym-Cym-Alm-(4←1)-Glc (type s-**4**) (Table 3). Glycosides **1** (hoyacarnoside A), **4** (hoyacarnoside D), **6**, **8** (hoyacarnoside F), **12** (hoyacarnoside I), **15** (hoyacarnoside L), **17** (hoyacarnoside M), **19** (hoyacarnoside P) and **22** (hoyacarnoside S) were classified into type s-**1**, and **2** (hoyacarnoside B), **9** (hoyacarnoside G) and **13** (hoyacarnoside J) into type s-**2**. Glycosides belonging to type s-**3** were **3** (hoyacarnoside C), **5** (hoyacarnoside E), **7**, **10**, **14** (hoyacarnoside K), **16** (hoyacarnoside N), **18** (hoyacarnoside O), **20** (hoyacarnoside Q), **21** (hoyacarnoside R) and **23** (hoyacarnoside T), and the type s-**4** sequence was seen in **11** (hoyacarnoside H) only.

All pregnanes obtained by hydrolysis appeared in glycosides **1**—**23**, except for a-**11**. Types s-**1** and s-**3** were observed in almost all pregnanes (Chart 2).

On ^{1}H-NMR measurement of the pregnanes in CD_3OD, deuterization was observed in H-21 of some samples. Decreasing intensity or the disappearance of 21-proton signals was observed in a-**2**, a-**3**, a-**5**, a-**6**, a-**7** and their glycosides, **4**, **6**, **12**, **16** and **18**, all of which have 12β-*O*-acyl groups and a β-oriented 17-acetyl side chain, although a remarkable exchanging effect was not seen within two weeks in the case of a-**2**. When C-20 is a carbinol (a-**8**—a-**10**) or when a 17-acetyl side chain retains an α-orientation such as in ikemagenin or kidjolanin,[3,10] no substitution of a proton to deuterium occurred. In a-**1** and a-**4**, which have no acyl group at C-12, no substitution was seen.

In this study, pregnanes and pregnane glycosides from the caules were examined. The glycosides from the leaves are described elsewhere, along with minor glycosides from the caules. To our knowledge, sakuragenin is the first 19-ace-

Table 3. NMR Spectral Data for the Sugar Moieties (s-**1**—s-**4**) (δ ppm in Pyridine-d_5)

	s-**1**		s-**2**		s-**3**		s-**4**	
	C	H	C	H	C	H	C	H
	Cym		Cym		Cym		Cym	
1	96.2	5.28 (br d,10)	96.4	5.25 (br d, 10)	96.2	5.29 (dd, 9,2)	96.3	5.30 (br d, 10)
2	37.2	1.90, 2.31 (m)	37.2	1.89, 2.27 (m)	37.2	1.89,2.32 (m)	37.2	1.88, 2.30 (m)
3	78.0	4.07 (q, 3)	78.1	4.02 (q, 3)	78.0	4.08 (q, 3)	78.0	4.06 (q, 3)
4	83.3	3.58 (dd, 9,2)	83.3	3.44 (dd, 9,3)	83.3	3.48 (dd, 9,3)	83.3	3.47 (dd, 9, 3)
5	68.9[a)]	4.15 (m)	69.0[a)]	4.20 (m)	68.9[a)]	4.20 (m)	68.9	4.21 (m)
6	18.5	1.55 (d, 6)	18.5	1.35 (d, 6)	18.5	1.34 (d, 6)	18.5	1.34 (d, 6)
	Cym		Cym		Cym		Cym	
1	100.4	5.09 (dd, 9,2)	100.3	5.09 (dd, 9,2)	100.4	5.09 (dd, 10,2)	100.3	5.09 (br d, 10)
2	37.0	1.79, 2.28 (m)	37.0	1.80, 2.30 (m)	37.0	1.79, 2.27 (m)	37.0	1.75, 2.20 (m)
3	77.6	3.99 (q, 3)	77.9	4.04 (q, 3)	77.7	3.99 (q, 3)	78.1	3.99 (q, 3)
4	83.1	3.42 (dd, 9,3)	83.2	3.47 (dd, q, 3)	83.1	3.41 (dd, 9,3)	83.2	4.14 (dd, 9,3)
5	68.8[a)]	4.14(m)	69.2[a)]	4.16 (m)	68.8[a)]	4.14 (m)	68.9	4.13 (m)
6	18.4	1.37 (d, 6)	18.4	1.44 (d, 6)	18.4	1.36 (d, 6)	18.4	1.31 (d, 6)
	Ole		Alm		Ole		Cym	
1	101.8	4.67 (dd, 9,2)	103.9	5.07 (d, 8)	101.8	4.66 (dd, 10,2)	100.3	5.07 (br d, 10)
2	37.5	1.72, 2.47 (m)	72.4	3.79 (br d, 8)	37.5	1.70, 2.46 (m)	37.3	1.80, 2.32 (m)
3	79.2	3.50-3.62	83.0	4.44 (t, 3)	79.2	3.50-3.60	78.2	4.05 (q, 3)
4	82.7	3.60 (t, 9)	83.0	3.72 (dd, 9,3)	82.8	3.55 (t, 9)	83.0	3.47 (dd, 9,3)
5	72.0	3.52-3.62	69.2	4.16 (m)	71.9	3.50-3.60	69.2	4.18 (m)
6	18.8	1.63(d, 6)	18.2	1.61 (d, 6)	18.8	1.59 (d, 6)	18.3	1.45 (d, 6)
	Alm		Glc		Alm		Alm	
1	101.9	5.28 (d, 8)	106.5	4.97 (d, 8)	101.8	5.25 (d, 8)	103.9	5.08 (d, 8)
2	73.2	3.87 (br d, 8)	75.4	4.02(dd, 8,9)	72.6	3.81 (br d, 8)	72.4	3.80 (dd, 8,3)
3	83.9	4.07 (t, 3)	78.3	4.24 (t, 9)	83.1	4.47 (t, 3)	83.0	4.45 (t, 3)
4	74.5	3.58 (dd, 8,3)	71.9	4.29 (t, 9)	83.3	3.73 (dd, 9,3)	83.0	3.72 (dd, 9,3)
5	71.0	4.15 (m)	78.3	3.98 (m)	69.5	4.25 (dq, 9,6)	69.2	4.26 (dq, 9,3)
6	18.5	1.55 (d, 6)	63.0	4.36 (dd, 12,5)	18.2	1.64 (d, 6)	18.2	1.62 (d, 6)
				4.52 (dd, 12,2)				
					Glc		Glc	
1					106.5	4.96 (d, 8)	106.5	4.97 (d, 8)
2					75.4	4.01 (dd, 8,9)	75.4	4.03 (dd, 8,9)
3					78.3	4.23 (t, 9)	78.3	4.26 (t, 9)
4					71.9	4.18 (t, 9)	71.9	4.20 (t, 9)
5					78.0	3.97 (m)	78.3	3.98 (m)
6					63.0	4.35 (dd, 12,5)	63.0	4.37 (dd, 12,5)
						4.52 (dd, 12,2)		4.53 (dd, 12,3)
OMe	57.1	3.52 (s)	58.8	3.61 (s)	57.3	3.51 (s)	58.8	3.61 (s)
	58.8	3.55 (s)	59.0	3.58 (s)	58.8	3.54 (s)	58.9	3.62 (s)
	58.8	3.63 (s)	61.7	3.86 (s)	58.8	3.63 (s)	59.0	3.57 (s)
	62.0	3.83 (s)			61.6	3.83 (s)	61.7	3.84 (s)

a) Signal assignment may be interchangeable.

toxylated pregnane from a plant source. Its glycosides, however, have not yet been obtained.

Experimental

Melting points were taken on a hot stage apparatus without correction. ^{1}H- and ^{13}C-NMR spectra were measured by a JNM-A500 spectrometer in CD_3OD or pyridine-d_5, unless otherwise noted. Chemical shifts are given in δ values, relative to internal tetramethylsilane, and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=doublet of doublets, br=broad. HR-FAB-MS were recorded on a JEOL HX-110 spectrometer. Optical rotations are measured on a JASCO-DIP 360 polarimeter. The following solvent systems were used for column chromatography and TLC: 1, $CHCl_3$–MeOH–H_2O (10 : 1 : 1.2—7 : 3 : 1.6, bottom layer); 2, EtOAc–MeOH–H_2O (8 : 1 : 1.2—6 : 1 : 1.2, top layer); 3, benzene-acetone (5 : 1—1 : 1). Spray reagent for TLC, 10% H_2SO_4.

Plant Materials *H. carnosa* R. Br. was collected in Okinawa in October, 1995, and the leaves were removed from the caules and soaked in MeOH. The caules (4.5 kg) were cut and air-dried (dried weight, 1.7 kg). The dried caules were powdered and percolated with MeOH at room temperature. The whole MeOH solution was concentrated *in vacuo* and partitioned with $CHCl_3$. A portion (8 g) of the $CHCl_3$ extract (29 g) was purified on a silica gel column with solvent 1, and the fraction containing pregnane glycosides was subjected to hydrolysis with 0.05 N HCl–50% dioxane (80 ml) at 95 °C for 2 h. After deacidification with Amberlite IRA 410, the solution was concentrated *in vacuo* and partitioned with $CHCl_3$ to separate pregnanes ($CHCl_3$ layer) and sugars (H_2O layer).

Pregnanes The pregnane fraction was chromatographed on a silica gel column with solvent 1 to give eleven pregnanes (a-**1**—a-**11**), in which five were identified as drevogenin P (a-**1**), drevogenin A (a-**3**), 17β-marsdenin (a-**4**), drebyssogenin J (a-**8**) and marsectohexol (a-**9**), by comparisons of MS and NMR data with those in the literature. (a-**1**, 9 mg; a-**2**, 2 mg; a-**3**, 22 mg, a-**4**, 23 mg; a-**5**, 31 mg; a-**6**, 22 mg; a-**7**, 15 mg; a-**8**, 31 mg; a-**9**, 2 mg; a-**10**, 18 mg; a-**11**, 7 mg).

11,12-Di-*O*-acetyldrevogenin P (a-**2**): Solid, $[\alpha]_D^{23}$ +32.0° (c=0.35, MeOH). HR-FAB-MS *m/z*: 471.2359 (Calcd for $C_{25}H_{36}O_7$ +Na: 471.2358).

11,12-Di-*O*-acetyl-17β-marsdenin (a-**5**): Solid, $[\alpha]_D^{22}$ +76.2° (c=0.98, MeOH). HR-FAB-MS *m/z*: 487.2309 (Calcd for $C_{25}H_{36}O_8$ +Na: 487.2308). HR-FAB-MS of 21-D_3 form *m/z*: 490.2497 (Calcd for $C_{25}H_{33}D_3O_8$+Na: 490.2496).

8-Hydroxydrevogenin A (a-**6**): (21-D_3 form) Fine prisms (MeOH), mp 174—176 °C , $[\alpha]_D^{26}$ +71.7° (c=1.13, MeOH). FAB-MS *m/z*: 532.2967 (Calcd for $C_{28}H_{39}D_3O_8$ +Na: 532.2966).

5,6-Didehydrocynafogenin (a-**7**): (21-D_3 form) Fine prisms (MeOH), mp 131—133 °C , $[\alpha]_D^{30}$ +110.9° (c=0.59, MeOH). FAB-MS *m/z*: 552.2651 (Calcd for $C_{30}H_{35}D_3O_8$+Na: 552.2653).

11,12-Di-*O*-acetylmarsectohexol (a-**10**): Solid, $[\alpha]_D^{21}$ +31.8° (c=0.95,

MeOH). FAB-MS *m/z*: 489.2465 (Calcd for $C_{25}H_{38}O_8$ +Na: 489.2464).

Sakuragenin (a-**11**): Needles (MeOH), mp 225—229 °C, $[\alpha]_D^{26}$ −16.8° (*c*=0.31, MeOH). FAB-MS *m/z*: 429.2253 (Calcd for $C_{23}H_{34}O_6$+Na: 429.2254).

Sugars After separation of the pregnanes, the H_2O layer was concentrated *in vacuo* to dryness and the residue was chromatographed on a silica gel column with solvent 1 to give D-cymarose (46 mg,$[\alpha]_D^{26}$ +52°, H_2O, 24 h), D-oleandrose (35 mg, $[\alpha]_D^{26}$ −10°, H_2O, 24 h), 6-deoxy-3-*O*-methyl-D-allose (31 mg, $[\alpha]_D^{26}$ +8°, H_2O, 24 h), and D-glucose (7 mg, $[\alpha]_D^{26}$ +56°, H_2O, 24 h).

Isolation of Pregnane Glycosides A portion of the $CHCl_3$ extract (21 g) was chromatographed on a silica gel column with solvents 1 and 2, an ODS column with MeCN–H_2O (30—80%), and finally on HPLC (ODS column) with MeCN–H_2O (40—65%) to isolate 23 glycosides, **1**, 97 mg; **2**, 14 mg; **3**, 40 mg; **4**, 22 mg; **5**, 63 mg; **6**, 21 mg; **7**, 32 mg; **8**, 202 mg; **9**, 12 mg; **10**, 76 mg; **11**, 9 mg; **12**, 47 mg; **13**, 16 mg; **14**, 103 mg; **15**, 26 mg; **16**, 36 mg; **17**, 17 mg; **18**, 27 mg; **19**, 26 mg; **20**, 107 mg; **21**, 9 mg; **22**, 12 mg; **23**, 34 mg. Among these, three glycosides, dregeoside A_{p1} (**6**), dregeoside A_{o1} (**7**) and condurangoside D_{o1} (**10**), were known. Glycoside **8** was most major of all the glycosides, and **1**, **14** and **20** were obtained in high yields.

Hoyacarnoside A (**1**): Solid, $[\alpha]_D^{26}$ +14.1° (*c*=1.53, MeOH). HR-FAB-MS *m/z*: 979.5237 (Calcd for $C_{49}H_{80}O_{18}$+Na: 979.5243).

Hoyacarnoside B (**2**): Solid, $[\alpha]_D^{28}$ +15.0° (*c*=0.68, MeOH). HR-FAB-MS *m/z*: 997.4986 (Calcd for $C_{48}H_{78}O_{20}$+Na: 997.4984).

Hoyacarnoside C (**3**): Solid, $[\alpha]_D^{25}$ +15.3° (*c*=1.21, MeOH). HR-FAB-MS *m/z*: 1141.5774 (Calcd for $C_{55}H_{90}O_{23}$+Na: 1141.5771).

Hoyacarnoside D (**4**): Solid, $[\alpha]_D^{33}$ +36.6° (*c*=1.20, MeOH). HR-FAB-MS *m/z*: 1063.5457 (Calcd for $C_{53}H_{84}O_{20}$+Na: 1063.5454).

Hoyacarnoside E (**5**): Solid, $[\alpha]_D^{26}$ +28.6° (*c*=1.48, MeOH). HR-FAB-MS *m/z*: 1225.5989 (Calcd for $C_{59}H_{94}O_{25}$+Na: 1225.5982).

Hoyacarnoside F (**8**): Needles, mp 244—249 °C, $[\alpha]_D^{26}$ +31.2° (*c*=1.80, MeOH). HR-FAB-MS *m/z*: 995.5194 (Calcd for $C_{49}H_{80}O_{19}$+Na: 995.5191).

Hoyacarnoside G (**9**): Solid, $[\alpha]_D^{28}$ +41.1° (*c*=0.81, MeOH). HR-FAB-MS *m/z*: 1013.4949 (Calcd for $C_{48}H_{78}O_{21}$+Na: 1013.4933).

Hoyacarnoside H (**11**): Solid, $[\alpha]_D^{33}$ +26.4° (*c*=0.45, MeOH). HR-FAB-MS *m/z*: 1157.5721 (Calcd for $C_{55}H_{90}O_{24}$+Na: 1157.5719).

Hoyacarnoside I (**12**): Solid, $[\alpha]_D^{27}$ +42.3° (*c*=2.25, MeOH). HR-FAB-MS *m/z*: 1079.5406 (Calcd for $C_{53}H_{84}O_{21}$+Na: 1079.5402).

Hoyacarnoside J (**13**): Solid, $[\alpha]_D^{27}$ +35.1° (*c*=1.03, MeOH). HR-FAB-MS *m/z*: 1097.5149 (Calcd for $C_{52}H_{82}O_{23}$+Na: 1097.5145).

Hoyacarnoside K (**14**): Solid, $[\alpha]_D^{24}$ +31.5° (*c*=2.03, MeOH). HR-FAB-MS *m/z*: 1241.5929 (Calcd for $C_{59}H_{94}O_{26}$+Na: 1241.5931).

Hoyacarnoside L (**15**): Solid, $[\alpha]_D^{28}$ +52.5° (*c*=1.80, MeOH). HR-FAB-MS *m/z*: 1121.5874 (Calcd for $C_{56}H_{90}O_{21}$+Na: 1121.5872).

Hoyacarnoside M (**16**): Solid, $[\alpha]_D^{26}$ +39.9° (*c*=1.89, MeOH). HR-FAB-MS *m/z*: 1283.6401 (Calcd for $C_{62}H_{100}O_{26}$+Na: 1283.6400).

Hoyacarnoside N (**17**): Solid, $[\alpha]_D^{29}$ +75.9° (*c*=1.21, MeOH). HR-FAB-MS *m/z*: 1141.5564 (Calcd for $C_{58}H_{86}O_{21}$+Na: 1141.5559).

Hoyacarnoside O (**18**): Solid, $[\alpha]_D^{25}$ +56.4° (*c*=1.40, MeOH). HR-FAB-MS *m/z*: 1303.6082 (Calcd for $C_{64}H_{96}O_{26}$+Na: 1303.6088).

Hoyacarnoside P (**19**): Solid, $[\alpha]_D^{27}$ +14.3° (*c*=1.80, MeOH). HR-FAB-MS *m/z*: 1065.5612 (Calcd for $C_{53}H_{86}O_{20}$+Na: 1065.5611).

Hoyacarnoside Q (**20**): Solid, $[\alpha]_D^{27}$ +13.9° (*c*=2.25, MeOH). HR-FAB-MS *m/z*: 1227.6123 (Calcd for $C_{59}H_{96}O_{25}$+Na: 1227.6138).

Hoyacarnoside R (**21**): Solid, $[\alpha]_D^{28}$ +14.7° (*c*=0.64, MeOH). HR-FAB-MS *m/z*: 1159.5872 (Calcd for $C_{55}H_{92}O_{24}$+Na: 1159.5877).

Hoyacarnoside S (**22**): Solid, $[\alpha]_D^{28}$ +29.4° (*c*=0.85, MeOH). HR-FAB-MS *m/z*: 1081.5544 (Calcd for $C_{53}H_{86}O_{21}$+Na: 1081.5559).

Hoyacarnoside T (**23**): Solid, $[\alpha]_D^{26}$ +16.3° (*c*=2.10, MeOH). HR-FAB-MS *m/z*: 1243.6086 (Calcd for $C_{59}H_{96}O_{26}$+Na: 1243.6087).

Examination of Deuterization on Other Steroids Five—ten mg each of a-**1**, a-**4**, a-**8**, a-**9**, a-**10**, ikemagenin (12-*O*-cinnamoyl-3β, 8β, 12β, 14β-tetrahydroxy-17α-pregn-5-en-20-one), kidjolanin (12-*O*-cinnamoyl-3β, 8β, 12β, 14β, 17β-pentahydroxypregn-5-en-20-one), 12-*O*-acetyldigoxigenin, neridienone A (12β-hydroxypregna-4,6,16-triene-3,20-dione) was dissolved in CD_3OD (0.6 ml) and ^{1}H-NMR was measured after 2—3 weeks, but no H/D substitution reaction proceeded in each sample. Five mg each of **4**, **6**, **12**, **16** and **18** was dissolved in CD_3OD (0.6 ml) and ^{1}H-NMR was measured after 2—3 weeks. Deuterization of 21-CH_3 was observed in all samples.

Acknowledgement We thank Mr. H. Hanazono of Fukuoka University for MS operations.

References

1) Kindl H., Hoffmann–Ostenhof O., *Phytochemistry*, **5**, 1091—1102 (1966).
2) Abe F., Yamauchi T., *Phytochemistry*, **31**, 2819—2820 (1992).
3) Abe F., Mori Y., Okabe H., Yamauchi T., *Chem. Pharm. Bull.*, **42**, 1777—1783 (1994).
4) *a*) Sauer H. H., Weiss E., Reichstein T., *Helv. Chim. Acta*, **48**, 857—878 (1965); *b*) *Idem*, *ibid*., **49**, 1632—1655 (1966); *c*) *Idem*, *ibid*., **49**, 1655—1662 (1966).
5) *a*) Hayashi K., Mitsuhashi H., *Chem. Pharm. Bull*., **16**, 2522—2524 (1968); *b*) Umehara K., Endoh M., Miyase T., Kuroyanagi M., Ueno A., *Chem. Pharm. Bull*., **42**, 611—616 (1994).
6) Bhatnagar A. S., Stocklin W., Reichstein T., *Helv. Chim. Acta*, **51**, 133—147 (1968).
7) Yoshimura S., Narita H., Hayashi K., Mitsuhashi H., *Chem. Pharm. Bull*., **33**, 2287—2293 (1985).
8) Kimura M., Hayashi K., Narita H., Mitsuhashi H., *Chem. Pharm. Bull*., **30**, 3932—3941 (1982).
9) Yoshimura S., Narita H., Hayashi K., Mitsuhashi H., *Chem. Pharm. Bull*., **31**, 3971—3983 (1983).
10) Yamagishi T., Mitsuhashi H., *Chem. Pharm. Bull*., **20**, 2070—2071(1972).