*Phytochemistry*, Vol. 31, No 3, pp. 1058–1060, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00


# THE PREGNANE GLYCOSIDE MARSDEKOISIDE A FROM *MARSDENIA KOI*

JIN-LAN YUAN, ZHI-ZHEN LU,* GUI-XIAN CHEN, WEI-PEI DING, BING-NAN ZHOU,*† CLEMENS A. J. ERDELMEIER,‡§ MATTHIAS O. HAMBURGER,‡‖ HARRY H. S. FONG‡ and GEOFFREY A. CORDELL†‡

Family Planning Research Institute, Tong-ji Medical University, Wuhan 430030, People's Republic of China; *Shanghai Institute of Materia Medica, Academy of Sciences, Shanghai, People's Republic of China; ‡Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, U.S.A.

(*Received in revised form* 3 *June* 1991)

**Key Word Index**—*Marsdenia koi*; Asclepiadaceae; pregnane glycoside; marsdekoiside A; structure elucidation.

**Abstract**—A new pregnane glycoside, marsdekoiside A was isolated from the stems of *Marsdenia koi* (Asclepiadaceae) and its structure was elucidated from chemical and spectral data as 12-cinnamoyl-dihydrosarcostin-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-*O*-β-D-oleandropyranosyl-(1→4)-*O*-β-D-cymaropyranoside.

## INTRODUCTION

An aqueous extract of the roots of *Marsdenia tenacissima* has been reported to be used as a fertility regulating agent [1], and an ethanolic extract of the roots of *M. thyrsiflora* showed both uterine stimulant and antifertility effects in rats [2, 3]. As a part of our program to examine plants for new anti-implantation agents, we examined the stems of *Marsdenia koi* Tsiang. In previous papers [4, 5], we have reported the isolation and the characterization of several triterpenoids from this source and we report here on the isolation and structure elucidation of a new pregnane glycoside, marsdekoiside A (**1**).

## RESULTS AND DISCUSSION

Marsdekoiside A (**1**), $C_{51}H_{78}O_{17}$ (FD-MS: *m/z* 962 $[M]^+$), was isolated by chromatography on silica gel normal and reversed phase columns. The isolate showed positive Liebermann–Burchard and Keller–Killiani tests. The $^1$H NMR spectrum of **1** showed methyl resonances at δ0.97 (3H, *s*, Me-19), 1.09 (3H, *d*, *J* = 6.3 Hz, Me-21), 1.53 (3H, *s*, Me-18), 1.23. 1.28 and 1.32 (each 3H, *d*, *J* = 6.3 Hz, Me of sugar moieties), suggesting that **1** is a steroid deoxyglycoside. Resonances at δ6.44 (2H, *d*, *J* = 16.2 Hz), 7.79 (2H, *d*, *J* = 16.2 Hz), 7.41 (3H, *m*) were also displayed, indicating the presence of a cinnamoyl ester in the molecule. Acetylation ($Ac_2O$–pyridine) afforded a triacetate {FAB-MS (Na+NOBA, 210°): *m/z* 1088 $[M]^+$, 245 [diacetyl 3-*O*-methyl-6-deoxy-allopyranosyl]$^+$}. Alkali hydrolysis with 5% $K_2CO_3$ in methanol–water (1:1) converted **1** to deacylmarsdekoiside A (**2**) and an aromatic acid identified as cinnamic acid (IR, $^1$H NMR and HPLC).

The $^1$H NMR spectrum of deacyl marsdekoiside A showed the presence of three deoxysugar moieties, and acid hydrolysis afforded a genin **3**, and sugars **4** and **5**. Comparison of its spectral and physical data with literature values [6–8], indicated the genin to be dihydrosarcostin (**3**). The sugars were identified as D-cymarose (**4**) and D-pachybiose (**5**) by HPLC. The sugar sequences were determined by FD-MS fragmentation, viz. *m/z* 962 $[M]^+$, 800 $[M-(3\text{-}O\text{-methyl-6-deoxy-allopyranose})]^+$ and 511 $[M-305-147+H]^+$. From the *J*-values of the anomeric protons, all of the sugars have the β-configuration.

Based on the $^1$H–$^1$H COSY NMR spectra of **1** and **2**, it was shown that the H-12 resonance was shifted from δ4.66 in **1** to δ3.50 in **2**, suggesting that the cinnamoyl moiety is at C-12. Thus, the structure of marsdekoiside A (**1**) was established as 12-cinnamoyl-dihydrosarcostin-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-*O*-β-D-oleandropyranosyl-(1→4)-*O*-β-D-cymaropyranoside.

## EXPERIMENTAL

*General.* Mp: uncorr. NMR were measured at 300 or 400 MHz for $^1$H. TMS was used as int. std. TLC was performed on Si gel 60 $F_{254}$ and RP-$18_{254}$. Quindao Si gel (100–140 mesh) and LiChroprep RP-8 were used for CC.

*Plant material.* The stem material of *M. koi* was collected in Kwangdong Province, People's Republic of China. A herbarium specimen representative of the collection is deposited in the Family Planning Research Institute, Tong-ji Medical University, Wuhan, People's Republic of China.

*Isolation of marsdekoiside A* (**1**). Powdered and dried stems and leaves (6 kg) were percolated with MeOH at room temp. The crude extract (875 g) was chromatographed on Celite eluting with petrol, $CHCl_3$ and MeOH and from the $CHCl_3$ fraction (208 g), marsdekoiside A was isolated by repeated chromatography on silica gel and Lobar chromatography on a Yamazen Low Pressure Liquid Chromatography System with a RP-8 column.

*Properties of marsdekoiside A* (**1**). Amorphous powder, mp 166–168°, $[\alpha]_D$ + 22.7° (MeOH; *c* 0.22). IR $\nu_{max}^{KBr}$: 3400, 1710, 1635, 1170 cm$^{-1}$. UV $\lambda_{max}^{MeOH}$: 216 (log ε 4.08), 222 (4.01), 279 nm (4.15). $^1$H NMR (300 MHz, $CDCl_3$) δ0.97 (3H, *s*, Me-19), 1.09 (3H, *d*, *J*

†Author to whom correspondence should be addressed.
§Present address: Pharmazeutisches Institut, ETH Zentrum, CH-8092 Zürich, Switzerland.
‖Present address: Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie, Université de Lausanne, CH-1015 Lausanne, Switzerland.

= 6.3 Hz, Me-21), 1.23, 1.28, 1.32 (each 3H, *d*, *J* = 6.3 Hz, Me-6 of sugars), 1.53 (3H, *s*, Me-18), 3.43, 3.44, 3.66 (each 3H, *s*, OMe-3 of sugars). 4.31 (1H, *d*, *J* = 8.4 Hz, anomeric H), 4.66 (1H, *dd*, *J* = 11.4, 4.2 Hz, H-12), 4.77, 4.87 (each 1H, *dd*, *J* = 10, 2 Hz, anomeric H), 6.44 (1H, *d*, *J* = 16.2 Hz, α'-H), 7.41 (3H, *m*, H-3', H-4' and H-5'), 7.53 (2H, *m*, H-2' and H-6'), 7.79 (1H, *d*, *J* = 16.2 Hz, β'-H). $^{13}$C NMR (pyridine-$d_5$) δ: see Table 1. FD-MS *m/z*: 985 $[M+Na]^+$, 962 $[M]^+$, 800 $[M-(3\text{-}O\text{-methyl-6-deoxy-allose})]^+$, 657 $[M-\text{pachybiose}+2H]^+$, 511 $[M-305-147+H]^+$, 365 $[\text{genin}-18-H]^+$, 161 $[3\text{-}O\text{-methyl-6-deoxy-allosyl}]^+$.

*Alkali hydrolysis of compound* (**1**). A soln of **1** (100 mg) in MeOH was treated with 5% $K_2CO_3$ in aq. MeOH for 48 hr room temp. After evapn of MeOH, the deacyl glucoside **2** was obtained by extn with $CHCl_3$ (50 ml). The aq. layer after acidification and extn with ethyl ether (50 ml) afforded *trans*-cinnamic acid (9 mg) by UV, IR, EI-MS. Compound **2**, amorphous powder, mp 157–159°; IR $\nu_{max}^{KBr}$: 3400, 1150 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$) δ: 0.96 (3H, *s*, Me-19), 1.14 (3H, *d*, *J* = 6.3 Hz, Me-21), 1.20, 1.25, 1.29 (each 3H, *d*, *J* = 6.2, 6.2 and 6.4 Hz, respectively, Me-5' of sugars) 1.31 (3H, *s*, Me-18), 3.40, 3.41, 3.63 (each 3H, *s*, OMe-3 of sugars), 4.02 (1H, *q*, *J* = 6.3 Hz, H-20), 3.50 (1H, *dd*, *J* = 4.4, 11.3 Hz, H-12), 4.28 (1H, *d*, *J* = 7.6 Hz, anomeric H), 4.73, 4.83 (each 1H, *dd*, *J* = 10, 2 Hz, anomeric H).

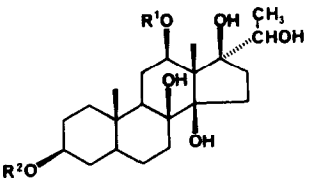
*Acid hydrolysis of compound* **2**. Compound **2** (59 mg) in 1% HCl–aq. MeOH soln (30%, 50 ml) was kept at 65° for 2 hr and extd with $CHCl_3$. The $CHCl_3$ ext was chromatographed on silica gel eluting with $CHCl_3$–MeOH (9:1) to afford **3** (11 mg), **4** and **5** (3 mg). Compound **3**. Prisms, mp 238–240°. IR $\nu_{max}^{KBr}$: 3560, 3260, 1105, 1045 cm$^{-1}$. $^1$H NMR (400 MHz, pyridine-$d_5$) δ: 1.24 (3H, *s*, Me-19), 1.48 (3H, *d*, *J* = 6.3 Hz, Me-21), 1.93 (3H, *s*, Me-18), 3.89 (2H, *m*, H-3 and H-12), 4.43 (1H, *q*, *J* = 6.3 Hz, H-20). $^{13}$C NMR (pyridine-$d_5$): see Table 1. EI-MS *m/z*: 366 $[M-H_2O]^+$, 348, 339, 330, 322, 321, 304, 303. The isolate was identified as dihydrosarcostin by direct comparison with an authentic sample. Compound **4**. The sugar was reduced with $NaBH_4$ and acetylated with $Ac_2O$–pyridine. GLC analysis (2% OV-1, col. temp. 210°, FID) indicated the same retention as the corresponding derivative of D-cymarose. Compound **5**. $^1$H NMR (400 MHz, $CDCl_3$–$CD_3OD$, 9:1) δ 1.14 (3H, *d*, *J* = 6.0 Hz, Me-5), 1.16 (3H, *d*, *J* = 6.3 Hz, Me-5'), 1.44 (1H, *ddd*, *J* = 13.7, 10.0, 2.5 Hz, H-2β), 2.02 (1H, *ddd*, *J* = 13.9, 3.8, 2.0 Hz, H-2a), 3.28 (3H, *s*, OMe-3), 3.50 (3H, *s*, OMe-3'), 4.14 (1H, *d*, *J* = 7.8 Hz, H-1'), 4.59 (1H, *dd*, *J* = 9.4, 2.0 Hz, H-1). The HPLC $R_f$ was the same as that of an authentic sample of pachybiose. EI-MS *m/z*: 322 $[M]^+$, 304, 286, 273, 258.

*Acknowledgements*—This work was supported, in part, by grants (HRP Projects 77918) to the Family Planning Research Institute, Yong-ji Medical University, Wuhan, People's Republic of China and Shanghai Institute of Materia Medica, Academia Sinica, People's Republic of China from the Task Force on Indigenous Plants for Fertility Regulation, Special Programme of Research, Development and Research Training in Human Reproduction,

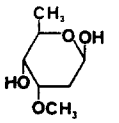
Table 1. Spectral data of compounds **1** and **2***

| C | Aglycone moiety 1 | 2 | C | Sugar moiety |
|---|---|---|---|---|
| 1 | 38.3 | 38.6 | Cym. 1 | 96.2 |
| 2 | 29.7 (−2.3) | 32.0 | 2 | 37.2 |
| 3 | 77.0 (+6.1) | 70.9 | 3 | 78.2 |
| 4 | 34.8 (−4.1) | 38.9 | 4 | 83.3 |
| 5 | 48.6 | 40.1 | 5 | 69.1 |
| 6 | 25.3 | 28.2 | 6 | 18.5 |
| 7 | 24.7 | 25.5 | 3-OMe | 58.9 |
| 8 | 76.0 | 76.1 | Ole. 1 | 100.5 |
| 9 | 47.1 | 47.6 | 2 | 37.5 |
| 10 | 36.6 | 36.6 | 3 | 78.3 |
| 11 | 34.8 | 34.9 | 4 | 83.4 |
| 12 | 76.0 | 72.9 | 5 | 70.7 |
| 13 | 57.7 | 59.2 | 6 | 18.7 |
| 14 | 88.9 | 89.0 | 3-OMe | 58.9 |
| 15 | 32.9 | 34.1 | All. 1 | 106.2 |
| 16 | 33.7 | 34.1 | 2 | 75.2 |
| 17 | 88.5 | 88.8 | 3 | 87.9 |
| 18 | 12.1 | 11.7 | 4 | 75.9 |
| 19 | 13.0 | 13.3 | 5 | 69.5 |
| 20 | 72.8 | 71.7 | 6 | 18.7 |
| 21 | 19.4 | 17.7 | 3-OMe | 60.8 |
| CO | 166.0 | | | |
| α' | 117.2 | | | |
| β' | 146.2 | | | |
| 1' | 133.9 | | | |
| 2',6' | 128.9 | | | |
| 3',5' | 128.3 | | | |
| 4' | 130.7 | | | |

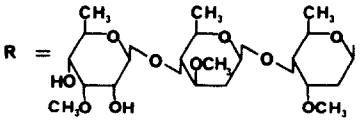
*δ values are given in ppm from internal TMS in pyridine-$d_5$. Glycosidation shifts are given in parentheses. Cym.: β-D-cymaropyranosyl; Ole.: β-D-oleandropyranosyl; All.: 3-*O*-methyl-6-deoxy-β-D-allopyranosyl.

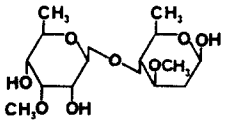


| | $R^1$ | $R^2$ |
|---|---|---|
| 1 | Ph CH=CH CO | R |
| 2 | H | R |
| 3 | H | H |



4





5

World Health Organization, Geneva, Switzerland. J.-L. Yuan and Z.-Z. Lu also acknowledge the World Health Organization for the award of Research Fellowships. We thank Prof. H. Mitsuhashi (Tsumura Juntendo Laboratory, Ibaraki, Japan) for authentic sugar samples. We also thank Prof. Y.-S. Gao (Honorary Director of Shanghai Institute of Materia Medica, Academia Sinica) for his continued interest and valuable discussion during the research.

## REFERENCES

1. Malhi, B. S. and Trivedi, V. P. (1972) *Quart J. Crude Drug Res.* **12**, 1922.
2. Chowdhury, A. K. A., Khaleque, R. A. and Khan, A. K. A. (1981) *Proc. Biochem. Silver Jub. Symp. and Conf.* Dacca, Bangladesh.
3. Chowdhury, A. K. A. and Khaleque, R. A. (1982) *Proc. 67th Bangladesh Sci. Conf.* Dacca, Bangladesh.
4. Yuan, J.-L., Ding, W.-P., Lu, Z.-Z., Shi, J.-P., Chen, H.-X. and Zhou, B.-N. (1991) *J. Tong-Ji Med. Univ.* **20**, 81.
5. Yuan, J.-L., Ji, Q., Chen, Q.-X. and Ding, W.-P. (1992) *Zhong Cao Yao* (in press).
6. Schaub, F., Kaufmann, H., Stocklin, W. and Reichstein, T. (1964) *Helv. Chim. Acta* **47**, 738.
7. Ardenne, M. V., Tummler, R., Weiss, E. K. and Reichstein, T. (1967) *Helv. Chim Acta* **50**, 1032.
8. Jaeggi, K. A., Weiss, E. K., Wehrli, W. and Reichstein, T. (1967) *Helv. Chim. Acta* **50**, 1201.