Chinese Chemical Letters Vol. 14, No. 5, pp 475 – 478, 2003
http://www.imm.ac.cn/journal/ccl.html

Cucurbitacin and Triterpenoid Glycosides from *Hemsleya giganthy*

Ya CHEN[2], Ming Hua CHIU[1]*, Kun GU[2], Zhong Rong LI[1]

[1]Laboratory of Phytochmistry, Kunming Institute of Botany , CAS, Kunming 650204
[2]Department of Chemistry, Yunnan University, Kunming 650091

Abstract: From rhizomes of *Hemsleya giganthy* collected in Shichuan of China, 16 compounds were isolated. Among them, three compounds (**8, 9, 15**) are new natural products called Hemslecins G; Hemsgiganosides A and B; respectively. Their structures were elucidated as 7-hydroxy-23, 24-dihydro-cucurbitacin F-25-O-acetate (**8**); 3-O-(6′-butyl ester-)-β-D-glu- curono-pyranosyl)-oleanolic acid-28-O-α-L-arabinopyranoside(**9**); 3-O-β-D-glucuropyranosyl oleanolic acid -28-O-β-D-gluco pyranosyl-(1→6)- β-D-glucopyranoside(**15**) by spectroscopic and chemical means.

Keywords: *Hemsleya giganthy*, Cucurbitaceae, Hemslecins G, Hemsgiganosides A, B.

Hemsleya gigantha is mainly distributed in southwestern part of China, especially abundant in Yunnan and Sichuan provinces. It has been said that this genus *Hemsleya* include thirty-one species until recent years[1]. This genus are known as herbal medicines in China, and have been used to cure bronchitis, bacillary dysentery, tuberculosis, diabetes, whooping cough and bile duct infection[2]. *Hemsleya gigantha* is a new species that comes from Sichuan province. In a serial studies on this species, four cucurbitacins[2] and two oleanane-type triterpenes[3] from *H. gigantha* were isolated. In the paper, we would like to present the isolation and structure elucidation of three new compounds called Hemslecins G; Hemsgiganosides A, B from the same plant.

Methanol extract of the rhizomes, harvested in Shimian county, Sichuan province of China, was suspended in water and the suspension was extract with ethyl acetate and 1-butanol, respectively. From the ether fraction, five known compounds have been isolated and elucidated in comparison TLC, ^{1}H NMR, ^{13}C NMR, IR and MS spectrums with that of authentic samples. They are β-amyrin[10] [**1**, white powder, 47 mg, $[\alpha]_D^{24}$ +39.3 (c 0.3, $CHCl_3$), mp:155-157°C, ^{13}C NMR data see **Table 2**]. Spinasterol (**2a**, white needle, 75mg, along with **2b**)[4,5], 22, 23-dihydrospinasterol (**2b**, white needle, 75 mg, along with **2a**)[4,5], spinasterol-3-O-β- D-glcoside(**3a**, white powder, 59 mg, along with **3b**)[4,5] and 22, 23-dihydrospinasterol-3-O-β-D-glcoside(**3b**, white powder, 59 mg, along with **3a**)[4,5] (**Figure 1**).

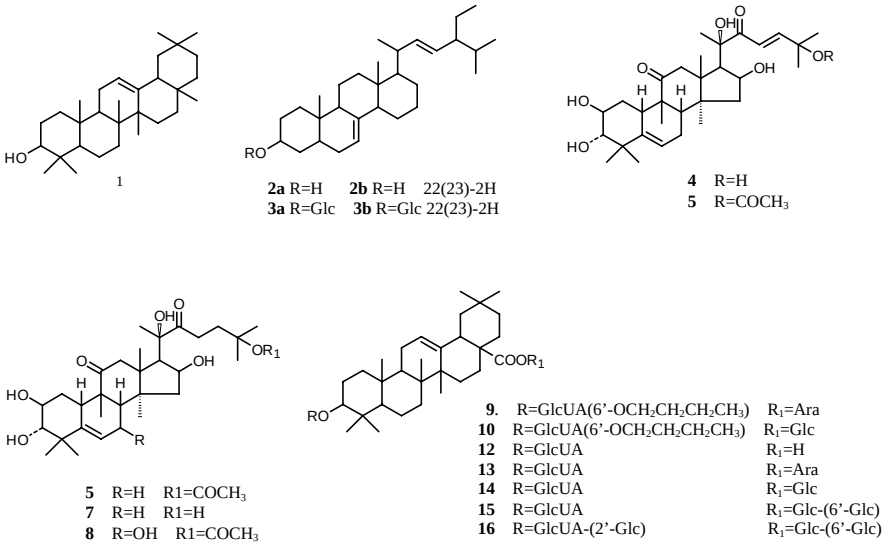
From the ethyl acetate fraction, four known cucurbitacins, cucurbitacin F(**4**), 23, 24-dihydrocucurbitacin F-25-O-acetate(**5**), cucurbitacin F-25-O-acetate(**6**), 23, 24-dihy-

*E-mail: mhchiu@public.km.yn.cn

drocucurbitacin F(**7**) and a new cucurbitacin: 7-hydroxy-23, 24-dihydro-cucurbtacin F-25-O-acetate(**8**) have been isolated. Compounds **4-7** were elucidated in comparison of ^{1}H NMR, ^{13}C NMR, IR and MS spectrums with that of authentic samples[2,6].

Compound **8** is a white powder. Its molecular ion peak in HRFAB-MS (negative) spectrum at *m/z* 577.3356 ([M-H]$^{-}$, calcd. 577.3376) suggested the molecular formula of **1** was $C_{32}H_{50}O_9$, which was confirmed by the ^{13}C NMR and DEPT . The IR spectrum showed the presence of hydroxy (3525 cm^{-1}) and acetoxy group (1705, 1251 cm^{-1})[8]. The ^{13}C NMR and DEPT of **8** exhibited 8 methine, 5 methylene, 9 methyl and 10 quaternary carbons, its ^{1}H NMR displayed 9 methyl signals at δ_H 1.28, 1.33, 1.47, 1.49, 1.50, 1.58, 1.62, 1.76, 1.87(s, 9xCH_3) and olefinic proton signal at δ_H 6.22(d, 1H, J=4.8Hz), these data suggested that compound 8 processes the skeleton of cucurbitacin F. The ^{13}C NMR signals of **8** at δc 122.5 (CH), 145.4 (C) and 215.3 (C), 217.1 (C) indicated the presence of a double oleafinic carbon and two ketone group. Comparison of the ^{13}C NMR spectrum of **8** with **5**, revealed that it possessed the same basic skeleton as **5**[8]. The difference between **8** and **5** was that **8** had a methine carbon that linked with hydroxyl (δc 66.29) and lacked a methylene carbon (compound **5**, C-7 at δc 23.5). We could infer that compound **8** has more one hydroxyl group at 7β than 23, 24-dihydro cucurbitacin F-25-O-acetate (**5**). The ^{1}H-^{1}H COSY spectra showed the proton at C-7 (δ_H 4.50) was interrelated to the proton at C-6 (δ_H 6.22) The HMBC spectra indicated that the proton of C-7 was interrelated to the C-5(δc 145.3), C-6(δc 122.5), C-9(δc 50.4), C-28(δc 19.7), C-29(δc 20.4). All of above results approved the hydroxy of C-7 is at β-position. Therefore, the structure of compound **8** was elucidated as 7-hydroxy-23, 24-dihydrocucurbitacin F-25-O-acetate.

Figure 1 Chemical structures of compounds **1-16**



1

2a R=H **2b** R=H 22(23)-2H
3a R=Glc **3b** R=Glc 22(23)-2H

4 R=H
5 R=$COCH_3$

5 R=H R1=$COCH_3$
7 R=H R1=H
8 R=OH R1=$COCH_3$

9. R=GlcUA(6'-$OCH_2CH_2CH_2CH_3$) R_1=Ara
10 R=GlcUA(6'-$OCH_2CH_2CH_2CH_3$) R_1=Glc
12 R=GlcUA R_1=H
13 R=GlcUA R_1=Ara
14 R=GlcUA R_1=Glc
15 R=GlcUA R_1=Glc-(6'-Glc)
16 R=GlcUA-(2'-Glc) R_1=Glc-(6'-Glc)

From the *n*-butanol fraction, eight compounds have been isolated. Four of them are known compounds, namely compounds **10(**80 mg**), 12(**33 mg**), 13(**373 mg**), 14(**134 mg**), 16(**1.26 g**)**[1,6,7] (**Figure 1**). These compounds were identified respectively as 3-O-(6'-butyl ester-)-β-D-glucurono pyra nosyl)- oleanolic acid- 28-O-β-D-glucopyranoside(**10**); oleanolic acid-3-O-β-D- glucuropyranoside (**12**); 3-O-β-D-glucuropyranosyl oleanolic acid- 28-O-α-L-arabinopyranoside(**13**); 3-O-β-D-glucuropy- ranosyl oleanolic acid -28-O-βD-glucopyranoside (**14**); 3-O-[β-D-gluco pyranosyl- (1→2)-β-D-glucuropyranosyl]-oleanolic acid-28-O-[β-D-glucopyranosyl (1→6)]- β-D-glucopyranoside(**16**) by comparison of their spectral data (MS,IR, ^{1}H NMR, ^{13}C NMR) with those of the authentic samples. The structure of **11**(8 mg) have not been identified. Compound **9**, **15** are two new triterpenoid saponins (**Figure 1**).

Compound **9** is a white powder. The molecular formula was assigned to be $C_{45}H_{72}O_{13}$ by HRFABMS(negative) at *m/z* 819.4825[M-H]$^-$ (calcd. 819.4895). On the FAB-MS (neg.) spectrum, the fragment ion peak at *m/z* 687 [M-1-132]$^-$ was a character fragment ion which lost arabinosyl moiety for molecular ion. On acidic hydrolysis, **9** gave oleanolic acid which were identified by TLC comparison with the authentic sample and glucurnic acid, arabinose in water layer that was identified by PC comparison with the authentic samples. Two carbon signals of **9** at δc 89.3 and 107.3 indicated presence of glucuronoside at C-3 of oleanolic acid [1].From the HMQC and ^{1}H NMR spectrum, we can conclude the anomeric proton signals of **9** at δ_H 4.97 (d, 1H, J=7.7Hz) in C_5D_5N as well as the anomeric carbon signal of **9** at δc 107.3 indicated that the anomeric configuration of the glucuronide was β-linked. Alkaline saponification of **9** gave arabinose in the water layer that was identified by TLC comparison with authentic sample. The anomeric carbon signals of **9** at δc 95.7 and 176.6 indicated the presence of 28-arabinopyranoside in oleanolic acid. In ^{1}H NMR, the proton signal at δ_H 6.26 (d, J=5.8Hz) indicated that the configuration of arabinopyranose is the α-form. Comparing of the ^{13}C NMR spectra with **13**[9], we can find compound **9** has more four carbon signals at δc 13.8 (methyl carbon), 18.6 (methylene), 30.0 (methylene), and 65.0 (a methylene that attach to oxygen). The upfield shift (-8.34ppm) of C-6' (δc 170.3) of glucoside indicated that the carbon had formed to be an ester. Comparison of the FAB-MS (neg.), compound **9** has an ion peak at *m/z* 763 [M-1-$CH_2CH_2CH_2CH_3$]$^-$. These results led to the structure of **9** can be assigned as 3-O-(6'-butyl ester-)-β-D-glucuronopyranosyl)-oleanolic acid- 28-O-α-L-arabinopyranoside(**9**).

Compound **15** is a white powder. On acid hydrolysis, **15** gave oleanolic acid which were identified by TLC comparison with the authentic sample and glucose, glucuronic acid in the water layer that was identified by PC comparison with the authentic samples. On the FAB-MS(neg.) spectrum, the molecular ion peak at *m/z* 955[M-1]$^-$ and other fragment ion peaks at *m/z* 793[M-1-162]$^-$, 631[M-1-162-162]$^-$, 455[M-1-162-162-176]$^-$ indicated that **15** contains three glucosyl units and a glucurnic acid. Its molecular formula of $C_{48}H_{76}O_{19}$ was established by negative ion HRFABMS (found 955.4870[M-H]$^-$, calcd. 955.4902). Comparison of the ^{13}C NMR spectrum of **15** with that of oleanolic acid, revealed that **15** processed the same basic skeleton as oleanolic acid. Two carbon signals of **15** at δc 88.2, 106 indicated 3-linked glucurnide of oleanolic acid[1]. By comparison of ^{13}C NMR data of **15** with that of like-compounds[1,7], it was revealed the

presence of β-D-glucopyranosyl-(6→1)- β-D-glucopyranoside linked at the C-28 of oleanolic acid[10]. Based on these results, the structure of compound **15** was determined as 3-O-β-D-glucuropyranosyl oleanolic acid -28-O-β-D-glucopyranosyl-(1→6)- β-D-glucopyranoside.

Acknowledgments

The authors are grateful to the financial support of the National Natural Science Foundation of China (Grant No.39970086) and Natural Science Foundation of Yunnan (Grant No.98C089M), also thank the members of analytical group in Phytochemistry Laboratory, Kunming Institute of Botany for their measuring spectral data.

References and Notes

1. R. L. Nie, T. Morita , R. Kasai , J. Zhou , C. Y. Wu, O. Tanaka, *Planta medica*, **1984**, *50*, 322
2. P. Q. Yang , H. Y. Wang , W. J. Chang , C. T. Che, *Planta Medica*, **1988**, *54*, 349.
3. Y. Q. Shi, P. Q. Yang, H. Y. Wang, W. J. Chang , *West China Univ. Med. Sci.* **1995**, *10* (2), 90.
4. J. Fan, B. S. Feng, M. H. Qiu, R. L. Nie, *Acta Botanica Yunnanica*, **1988**, *10* (4), 475.
5. L. S. Ding, Y. Z. Chen, F. E. Wu, *China Journal of Chinese Materia Medica.* **1991**, *16* (5)，289.
6. Y. K. Yang, C. W. Gao, M. H. Qiu, R. L. Nie, *Acta Botanica Yunnanica*, **2000**, *22* (1):103.
7. Y. Q. Shi, P. Q. Yang, L. Chen, W. J. Chang, *Zhong Cao Yao(in Chinese)*, **1995**, *26* (12), 619.
8. Data of Hemslecins G **(8).** A white powder,$[\alpha]_D^{24}$ +114.2 (c 0.45, C_5D_5N). $C_{32}H_{50}O_9$, Negative ion HRFABMS *m/z*: found 577.3356($[M-H]^-$)(calcd 577.3376 for $C_{32}H_{49}O_9$). Mp; 132-138°C, IR ν, KBr: 3525, 2872, 2890, 1705, 1426, 1369, 1269, 1176, 1119, 1059, 1014, 951, 870, 680. UV $\lambda_{max}^{CHCl_3}$ nm: 203.5, 227.5. ^{1}H NMR (400MHz, C_5D_5N) δ_H : 6.22(d, 1H, J=4.8Hz, 6-H), 4.94(1H,m,2-H), 4.50(brd, 1H, J=4.8Hz, 7-H), 4.18(m,1H,16-H), 3.51(d, 1H, J=8.8Hz,3-H), 1.28, 1.33, 1.47, 1.49, 1.50, 1.58, 1.62, 1.76, 1.87（s,27H, 9×CH_3）. ^{13}C NMR（C_5D_5N, 100.6MHz）(listed in Table 1), FAB-MS（neg.）*m/z*(%): 577($[M-H]^-$,100), 560($[M-H_2O]^-$,12). EI-MS: 500, 421, 403, 358, 367, 309, 205, 187, 173, 161, 142, 113, 95, 69. ^{13}C NMR δc（C_5D_5N, 100.6 MHz）C-1-C-30: 34.7, 70.5, 81.3, 43.0, 145.3, 122.5, 66.3, 35.5, 50.4, 53.2, 215.2, 49.2, 47.9, 50.4, 46.6, 71.0, 59.1, 22.9, 15.6, 80.2, 25.4, 217.1, 32.3, 35.5, 81.8, 26.0, 26.1, 19.7, 20.4, 25.6; Ac: 170.3, 22.3.
9. Data of Hemsgiganosides A **(9).** A white powder,$[\alpha]_D^{26}$ +8.98 (c 2.05, C_5D_5N). $C_{45}H_{72}O_{13}$, negative ion HRFABMS *m/z*: found 819.4825($[M-H]^-$) (calcd. 819.4895 for $C_{45}H_{71}O_{13}$), mp: 271-273°C（decomposed）.IR ν, KBr 3490, 2940, 1730, 1388, 1364, 1259, 1163，1027, 950, 826, 776, 749, 631, 599. FAB-MS(neg.) *m/z* (%): 819($[M-H]^-$,6), 763($[M-CH_2CH_2CH_2CH_3]^-$,11), 687（($[M-H-Ara]^-$,100), 627(10), 455(21), 143(43). ^{1}H NMR (C_5D_5N, 400MHz) δ_H : 6.25(d, 1H, J=5.8Hz,Ara-1-H), 5.40(brs, 1H, 12-H), 4.96(d, 1H, J=7.7Hz, GlcUA-1-H), 4.23(t, 2H, J=6.0Hz,$COOCH_2$), 1.26,1.23, 1.00, 0.94, 0.93, 0.86, 0.743(s, 21H, 7xCH_3),0.73 (t, 3H, J=7.2Hz, $CH_2\underline{CH_3}$). ^{13}C NMR δc（C_5D_5N, 100.6 MHz）C-1-C-30: 38.6, 27.2, 79.0, 37.1, 55.2, 18.4, 34.7, 39.7, 47.6, 37.1, 23.7, 121.7, 145.2, 42.0, 27.2, 25.9, 47.7, 42.2, 47.9, 31.5, 34.3, 33.3, 28.4, 17.1, 15.6, 16.8, 26.9, 32.6, 33.3, 25.9; 3-GlcUA: 107.3, 75.5, 78.1, 73.1, 77.4, 170.3; O(CH_2)CH_3: 65.0, 30.0, 18.6, 13.8; 28-Ara: 95.7, 71.4, 73.9, 66.0, 66.1.
10. Data of Hemsgiganosides B**(15),** A white powder, $C_{48}H_{76}O_{19}$. negative ion HRFABMS *m/z*: found 955.4870($[M-H]^-$) (calcd. 955.4902 for $C_{48}H_{75}O_{19}$). FAB-MS(neg.) *m/z* (%): 955 ($[M-1]^-$,100) 793($[M-1-162]^-$, 8), 631 ($[M-1–162-162]^-$,33), 455($[M-1-162-162-176]^-$,7), 383(5), 159(10), 87(23). ^{1}H NMR (C_5D_5N, 400MHz) δ_H : 6.15(d, 1H,J=8.2Hz, 28-Glc-1-H), 5.37(brs, 1H, 12-H), 4.94(d, 1H, J=5.4Hz ,Ara-1-H), 4.22(d, 1H, J=8.5Hz, 1H, 6'-Glc-1-H), 1.24, 1.22, 1.11, 1.03, 0.84, 0.84, 0.81(s, 21H, 7xCH_3). ^{13}C NMR δc（C_5D_5N, 100.6 MHz）C-1-C-30: 37.8, 25.6, 88.2, 38.5, 54.9, 17.5, 32.1, 38.9, 47.0, 35.9, 22.7, 121.8, 143.2, 40.7, 25.6, 22.4, 45.3, 41.2, 46.1, 29.7, 33.0, 32.1, 27.2, 16.0, 14.6, 25.1, 175.6, 31.5, 22.7; 3-GlcUA: 106.0, 77.2, 76.6, 72.8, 77.6, 171.6; 28-Glc-Glc: 94.6, 74.4, 76.6, 70.9, 77.2, 68.4; 104.0, 77.0, 76.2, 70.5, 77.1, 61.6.

Received 7 June, 2002