Pubescone, a Novel 11(7 → 6)Abeo-14-norcarabrane Sesquiterpenoid from *Siegesbeckia pubescens*

by **Rui Wang**[a]), **Lei-Lei Liu**[b]), and **Yan-Ping Shi***[a])[b])

[a]) Key Laboratory of Chemistry of Northwestern Plant Resources & Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, P. R. China

[b]) State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, P. R. China

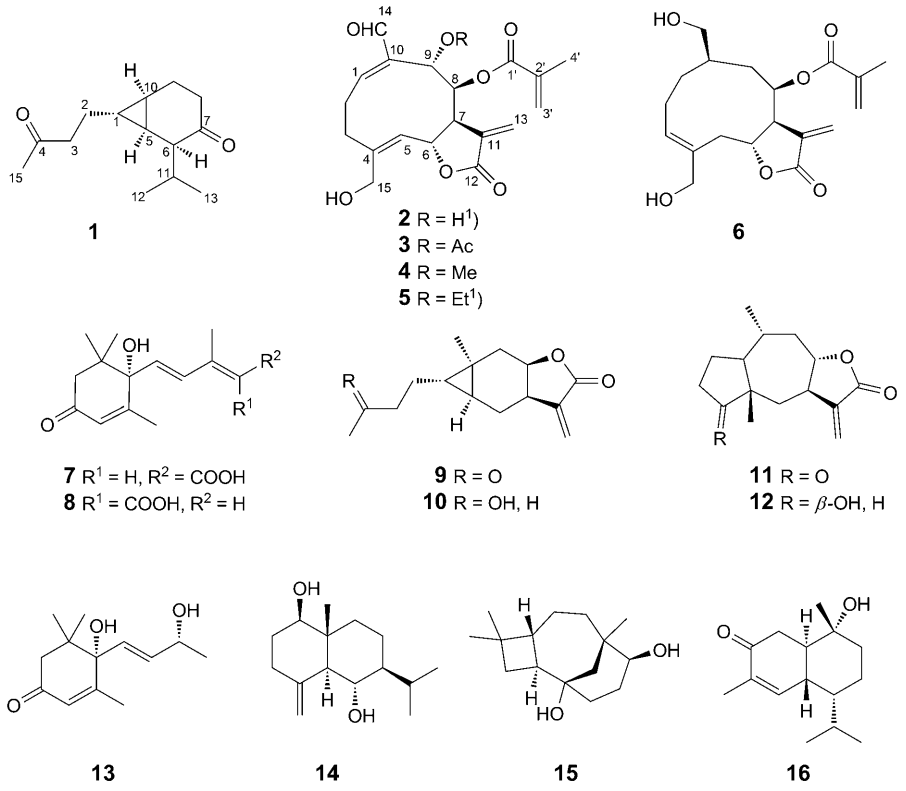
(phone: +86-931-4968208; fax: +86-931-8277088; e-mail: shiyp@lzb.ac.cn)

The phytochemical investigation of the aerial parts of *Siegesbeckia pubescens* L. yielded 16 sesquiterpenoids, including a novel 11(7 → 6)abeo-14-norcarabrane sesquiterpenoid, pubescone (**1**), and a new germacrane sesquiterpene, **5**. The molecular structures of the isolated compounds were elucidated on the basis of extensive spectroscopic analysis, including 1D- and 2D-NMR.

Introduction. – Plants of the genus *Siegesbeckia* (Asteraceae) are annual herbs widely distributed throughout tropical, subtropical, and temperate regions of the world. In China, the aerial parts of three species of *Siegesbeckia* have been used as the traditional Chinese medicine Xi-Xian to treat rheumatic arthritis, hypertension, malaria, neurasthenia, and snakebite [1]. Some chemical constituents of this genus have been proved to exhibit antioxidant [2], anti-allergic [3], and anti-infertility effects [4]. Series of diterpenes [1][5–10] and sesquiterpenes [11–13] from *Siegesbeckia* species have been identified previously. Quite recently, we have reported the isolation of 25 diterpenes from *Siegesbeckia pubescens* [14]. A further phytochemical investigation of this plant led to the isolation of 16 sesquiterpenoids, including a novel 11(7 → 6)abeo-14-norcarabrane skeletal sesquiterpenoid, **1**[1]), and a new germacrane sesquiterpene, **5**[1]). Additionally, to the best of our knowledge, compound **2** was obtained for the first time from nature. This report deals with the isolation and structure elucidation of these compounds.

Results and Discussion. – Compound **1**, a colorless oil, showed IR absorption bands at 1766 and 1717 cm^{-1}. The molecular formula was deduced to be $C_{14}H_{22}O_2$ from its HR-ESI-MS and ^{13}C-NMR spectra. The ^{1}H-NMR spectrum of **1** indicated the presence of three Me groups (δ(H) 2.18 (*s*, 3 H) and 0.92 (*d*, J = 6.8 Hz, 6 H)). The ^{13}C-NMR and DEPT spectra showed 14 C-signals, which were assignable to the presence of three Me, four CH_2, five CH, and two ketone C=O groups. All protons were assigned to the corresponding C-atoms by HMQC and ^{1}H,^{1}H-COSY experiments. Extensive analysis of the ^{1}H,^{1}H-COSY plot led to two substructures, **A** and **B** (*Fig. 1*), which were

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*



connected due to the HMBCs of Me(12) and Me(13) with C(6). The key HMBC correlations of Me(15) with C(3) and C(4) indicated the connection of C(3) and the C=O (C(4)) group. Taking into account the four degrees of unsaturation, the plane structure of **1** was assembled into a unique one. The relative configuration was determined by its NOESY data (*Fig. 2*). The NOE correlations between H–C(6) and H–C(10) and H–C(5) showed that they were all situated on the same side. The correlation of H–C(1) and H–C(11) indicated that H–C(1) was on the opposite side with respect to H–C(5). Accordingly, compound **1** was determined to be 11(7→6)abeo-14-norcarabran-4,7-dione possessing a novel C-skeleton, and **1** was named pubescone.

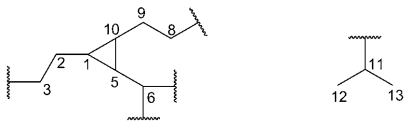


Fig. 1. *Partial structures from* $^1H,^1H$*-COSY plot for* **1**

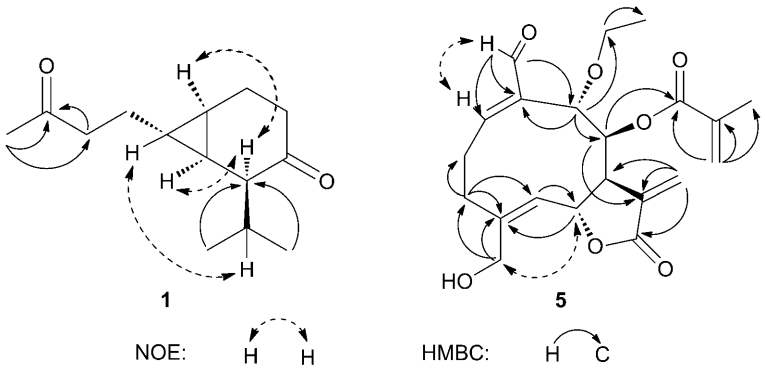


Fig. 2. *Selected NOE correlations and HMBCs for* **1** *and* **5**

Compound **2** was obtained as white needles. The NMR data suggested that **2** was almost identical to the known compound **4** [15], except for the absence of the oxygenated methyl group (δ(H) 3.18) in **4**. Compound **2** was thus determined to be (1(10)*E*,4*Z*,6α,8β,9α)-6,9,15-trihydroxy-8-(2-methylacryloxy)-14-oxogermacra-1(10),4,11(13)-trieno-12,6-lactone.

Compound **5** was obtained as colorless gum with the molecular formula $C_{21}H_{26}O_7$ as deduced by HR-ESI-MS and NMR analysis. The IR spectrum displayed the absorption bands due to OH groups (3423 cm^{-1}), a γ-lactone (1765 cm^{-1}), an unsaturated ester (1720 cm^{-1}), an α,β-unsaturated aldehyde (1685 cm^{-1}), and C=C bonds (1629 cm^{-1}). The NMR data of **5** were nearly superimposable with those of the known compound **4** [15], except for the presence of an EtO group at C(9) in **5** instead of the MeO group in **4**, which was confirmed by its HMBCs (*Fig. 2*). Therefore, the structure of **5** was determined to be (1(10)*E*,4*Z*,6α,8β,9α)-9-ethoxy-6,15-dihydroxy-8-(2-methylacryloxy)-14-oxogermacra-1(10),4,11(13)-trieno-12,6-lactone.

Other known 13 sesquiterpenoids isolated from this plant were orientalide (**3**) [11], pubetallin (**4**) [15], (3*E*,6α,8β)-6,14,15-trihydroxy-8-(2-methylacryloxy)germacra-3,11(13)- dieno-12,6-lactone (**6**) [16], (*E*,*E*)-abscisic acid (**7**) [17], (*Z*,*E*)-abscisic acid (**8**) [17], carabrone (**9**) [18], 4*H*-carabrone (**10**) [18], 2,3-dihydroaromaticin (**11**) [19], 2-deoxy-4-epipulchellin (**12**) [20], vomifoliol (**13**) [21], (1β,6α)-eudesm-4(14)-ene-1,6-diol (**14**) [22], (9β)-caryolane-1,9-diol (**15**) [23], and (10α)-hydroxyamorphan-4-en-3-one (**16**) [24]. Their structures were identified by comparison of their spectroscopic data with those reported in the literatures.

This work was financially supported by the *National Natural Science Foundation of China* (No. NSFC 20621091) and the *National Key Technology Research and Development Program of China* (No. 2007BAI37B05).

Experimental Part

General. Column Chromatography (CC): silica gel (200–300 mesh) from *Qingdao Marine Chemical Factory*, Qingdao, P. R. China. TLC: silica gel GF_{254} (10–40 μm) from *Qingdao Marine Chemical Factory*; Qingdao, P. R. China; detection at 254 nm and by spraying with 5% H_2SO_4/EtOH (*v*/*v*) followed by heating. Optical rotations: *Perkin-Elmer-341* polarimeter; 1 dm cell. UV Spectra: *NewCentury-*

Pgeneral T6 spectrophotometer; λ_{max} in nm. IR Spectra: *Nicolet-Nexus-670* FT-IR spectrometer; $\tilde{\nu}_{max}$ in cm^{-1}. NMR Spectra: *Varian-Inova-300* and *Bruker-Avance-III-400* spectrometers; chemical shifts δ in ppm rel. to Me_4Si as internal standard. HR-ESI-MS: *Bruker-Apex-II* mass spectrometer; in *m/z*.

Plant Material. The aerial parts of *Siegesbeckia pubescens* were purchased from the Huanghe Medicinal Material Market in Gansu in 2008, and were identified by associate Prof. *Huan-Yang Qi* at the Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics. A voucher specimen (No. ZY2007S001) was deposited with our laboratory.

Extraction and Isolation. The air-dried and powdered *S. Pubescens* plant material (9.0 kg) was extracted with EtOH (3×3 h) at *ca.* 65°. The crude extract was mixed with H_2O (2 l) to form a suspension, and then partitioned successively with petroleum ether, AcOEt, and BuOH. The AcOEt-soluble part (120 g) was subjected to CC (silica gel, petroleum ether/acetone 30 : 1, 15 : 1, 8 : 1, 4 : 1, 2 : 1, 1 : 1, and 0 : 100, and MeOH): *Fractions A–H*. *Fr. C* was separated by CC (silica gel, petroleum ether/AcOEt 30 : 1, 20 : 1, and 10 : 1, and MeOH), to give several subfractions. Further purification of each subfraction by repeated CC (petroleum ether/AcOEt, $CHCl_3$/AcOEt, and $CHCl_3$/acetone) yielded **1** (8.0 mg), **9** (15.1 mg), **10** (24.0 mg), **11** (4.0 mg), **14** (6.3 mg), and **16** (8.2 mg). *Fr. D* was subjected to CC (silica gel, $CHCl_3$/MeOH 50 : 1, 30 : 1, and 10 : 1, and MeOH), and then each subfraction was purified by CC (silica gel, $CHCl_3$/MeOH 50 : 1, $CHCl_3$/acetone 20 : 1, and $CHCl_3$/AcOEt 6 : 1): **3** (4.8 mg), **4** (3.0 mg), **5** (12.0 mg), **12** (11.4 mg), **13** (7.0 mg), and **15** (8.0 mg). A similar isolation procedure adopted for *Fr. E* afforded **2** (8.3 mg), **6** (3.5 mg), **7** (5.0 mg), and **8** (5.5 mg), resp.

Pubescone (= *11(7→6)Abeo-14-norcarabran-4,7-dione* = rel-*(1*R*,2*R*,6*S*,7*S*)-7-(3-Oxobutyl)-2-(1-methylethyl)bicyclo[4.1.0]heptan-3-one*; **1**): Colorless oil. $[\alpha]_D^{20} = -34$ ($c = 0.4$, acetone). IR (Film): 2956, 2874, 1766, 1717, 1182. ^{1}H-NMR ($CDCl_3$, 400 MHz): 2.53–2.48 (*m*, CH_2(3)); 2.18 (*s*, Me(15)); 2.14–2.11 (*m*, H_α–C(9)); 2.05 (*dd*, *J* = 8.8, 3.2, CH_2(8)); 2.02–1.99 (*m*, H_β–C(9)); 1.88–1.84 (*m*, H–C(10)); 1.80–1.77 (*m*, H–C(11)); 1.76–1.63 (*m*, CH_2(2)); 1.66–1.63 (*m*, H–C(1)); 1.05–1.03 (*m*, H–C(5)); 0.92 (*d*, *J* = 6.8, Me(12), Me(13)); 0.72–0.67 (*m*, H–C(6)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 214.2 (*s*, C(7)); 208.7 (*s*, C(4)); 46.4 (*d*, C(6)); 41.7 (*t*, C(3)); 34.4 (*d*, C(1)); 32.8 (*t*, C(8)); 30.9 (*d*, C(11)); 29.9 (*q*, C(15)); 29.9 (*d*, C(5)); 27.9 (*d*, C(10)); 24.8 (*t*, C(2)); 22.9 (*t*, C(9)); 19.5 (*q*, C(12)); 19.2 (*q*, C(13)). HR-ESI-MS (pos.): 223.1699 ($[M+H]^+$, $C_{14}H_{23}O_2^+$; calc. 223.1693).

*(1(10)*E*,4*Z*,6*α*,8*β*,9*α*)-6,9,15-Trihydroxy-8-(2-methylacryloxy)-14-oxogermacra-1(10),4,11(13)-trieno-12,6-lactone* (= rel-*(3a*R*,4*R*,5*R*,6*E*,10*Z*,11a*S*)-6-Formyl-2,3,3a,4,5,8,9,11a-octahydro-5-hydroxy-10-(hydroxymethyl)-3-methylidene-2-oxocyclodeca[*b*]furan-4-yl 2-Methylprop-2-enoate*; **2**): White needles. As a result of the instability and the limited amount of **2**, only the ^{1}H- and ^{13}C-NMR could be recorded. ^{1}H-NMR ((D_6)acetone, 400 MHz): 9.47 (*s*, H–C(14)); 6.79 (*dd*, *J* = 10.0, H–C(1)); 6.55 (*dd*, *J* = 8.4, 1.2, H–C(8)); 6.13 (br. *s*, H_a–C(3′)); 6.07 (*d*, *J* = 3.6, H_a–C(13)); 5.67 (*d*, *J* = 3.2, H_b–C(13)); 5.62 (br. *s*, H_b–C(3′)); 5.34 (*t*, *J* = 10.0, H–C(6)); 5.12 (*d*, *J* = 10.8, H–C(5)); 4.40 (*dd*, *J* = 13.2, CH_2(15)); 4.27 (*dd*, *J* = 8.4, 1.2, H–C(9)); 2.83–2.79 (*m*, H–C(7)); 2.83–2.79 (*m*, H_a–C(3)); 2.65–2.61 (*m*, H_a–C(2)); 2.59–2.55 (*m*, H_b–C(2)); 2.04–1.98 (*m*, H_b–C(3)); 1.92 (*s*, Me(4′)). ^{13}C-NMR ((D_6)acetone, 100 MHz): 196.5 (*s*, C(14)); 169.9 (*s*, C(12)); 167.1 (*s*, C(1′)); 156.7 (*d*, C(1)); 145.6 (*s*, C(4)); 142.3 (*s*, C(10)); 137.3 (*s*, C(2′)); 136.9 (*s*, C(11)); 128.6 (*d*, C(5)); 126.3 (*t*, C(3′)); 120.5 (*t*, C(13)); 74.8 (*d*, C(6)); 72.6 (*d*, C(8)); 69.4 (*d*, C(9)); 60.3 (*d*, C(15)); 51.9 (*d*, C(7)); 32.8 (*t*, C(3)); 27.6 (*t*, C(2)); 18.3 (*q*, C(4′)).

*(1(10)*E*,4*Z*,6*α*,8*β*,9*α*)-9-Ethoxy-6,15-dihydroxy-8-(2-methylacryloxy)-14-oxogermacra-1(10),4,11(13)-trieno-12,6-lactone* (= rel-*(3a*R*,4*R*,5*R*,6*E*,10*Z*,11a*S*)-5-Ethoxy-6-formyl-2,3,3a,4,5,8,9,11a-octahydro-10-(hydroxymethyl)-3-methylidene-2-oxocyclodeca[*b*]furan-4-yl 2-Methylprop-2-enoate*; **5**): Colorless gum. $[\alpha]_D^{20} = -9$ ($c = 1.2$, acetone). UV (acetone): 211. IR (KBr): 3423, 2970, 2931, 2874, 1765, 1720, 1685, 1629, 1148, 981. ^{1}H-NMR ($CDCl_3$, 300 MHz): 9.42 (*d*, *J* = 1.8, H–C(14)); 6.76 (*dd*, *J* = 9.9, H–C(1)); 6.48 (*dd*, *J* = 8.1, 1.2, H–C(8)); 6.15 (*d*, *J* = 3.6, H_a–C(13)); 6.05 (*s*, H_a–C(3′)); 5.81 (*d*, *J* = 3.0, H_b–C(13)); 5.52 (*s*, H_b–C(3′)); 5.20 (*dd*, *J* = 10.8, 9.9, H–C(6)); 4.96 (*d*, *J* = 10.5, H–C(5)); 4.36 (*dd*, *J* = 12.9, CH_2(15)); 3.93 (*dd*, *J* = 8.1, 1.8, H–C(9)); 3.34–3.28 (*m*, 1 H, MeCH_2O); 3.08–3.02 (*m*, 1 H, MeCH_2O); 2.77 (*dd*, *J* = 12.3, 2.1, H_a–C(2)); 2.70–2.64 (*m*, H_a–C(3)); 2.66–2.61 (*m*, H–C(7)); 2.60–2.51 (*m*, H_b–C(2)); 1.99–1.91 (*m*, H_b–C(3)); 1.88 (*s*, Me(4′)); 0.96 (*t*, *J* = 7.2, *Me*CH_2O). ^{13}C-NMR ($CDCl_3$, 75 MHz): 194.7 (*s*, C(14)); 169.6 (*s*, C(12)); 165.9 (*s*, C(1′)); 156.3 (*d*, C(1)); 141.1 (*s*, C(10)); 140.2 (*s*, C(4)); 135.8 (*s*, C(2′)); 134.0 (*s*, C(11)); 128.6 (*d*, C(5)); 125.8 (*t*, C(3′)); 122.1 (*t*, C(13)); 75.8 (*d*, C(9)); 73.9 (*d*, C(6)); 70.0 (*d*, C(8)); 64.3 (*t*, Me*C*H_2O); 60.5 (*t*, C(15)); 50.6 (*d*, C(7)); 32.5 (*t*, C(3)); 27.4 (*t*,

C(2)); 18.2 (*q*, C(4′)); 14.8 (*q*, *Me*CH_2O). HR-ESI-MS (pos.): 408.2012 ($[M+NH_4]^+$, $C_{21}H_{30}O_7N^+$; calc. 408.2017).

REFERENCES

[1] J. Xiong, Y. Ma, Y. Xu, *Phytochemistry* **1992**, *31*, 917.
[2] J. D. Su, T. Osawa, M. Namiki, *Agric. Biol. Chem.* **1986**, *50*, 199.
[3] H. M. Kim, C. Y. Kim, M. H. Kwon, T. Y. Shin, E. J. Lee, *Arch. Pharmacal. Res.* **1997**, *20*, 122.
[4] X. Y. Dong, M. Chen, W. Jin, D. X. Huang, S. M. Shen, H. T. Li, *Acta. Pharm. Sin.* **1989**, *24*, 833.
[5] Y. Xiang, H. Zhang, C. Q. Fan, J. M. Yue, *J. Nat. Prod.* **2004**, *67*, 1517.
[6] L. Canonica, B. Rindone, C. Scolastico, K. D. Han, J. H. Kim, *Tetrahedron Lett.* **1969**, *10*, 4801.
[7] J. H. Kim, K. D. Han, K. Yamasaki, O. Tanaka, *Phytochemistry* **1979**, *18*, 894.
[8] K. Liu, E. Roder, *Planta Med.* **1991**, *57*, 395.
[9] J. Xiong, Q. D. Jin, Y. L. Xu, *Chin. Chem. Lett.* **2001**, *12*, 51.
[10] H. Z. Fu, R. Feng, Z. H. Du, Z. C. Miu, X. X. Yan, Y. G. Li, *Zhongcaoyao* **1997**, *28*, 327.
[11] R. N. Baruah, R. P. Sharma, K. P. Madhusudanan, G. Thyagarajan, W. Herz, R. Murari, *Phytochemistry* **1979**, *18*, 991.
[12] C. Zdero, F. Bohlmann, R. M. King, H. Robinson, *Phytochemistry* **1991**, *30*, 1579.
[13] Y. Xiang, C.-Q. Fan, J.-M. Yue, *Helv. Chim. Acta* **2005**, *88*, 160.
[14] R. Wang, W. H. Chen, Y. P. Shi, *J. Nat. Prod.* **2010**, *73*, 17.
[15] R. N. Barua, R. P. Sharma, G. Thyagarajan, W. Herz, S. V. Govindan, *Phytochemistry* **1980**, *19*, 323.
[16] W. L. Chen, W. D. Tang, R. J. Zhang, L. G. Lou, W. M. Zhao, *J. Nat. Prod.* **2007**, *70*, 567.
[17] F. Ferreres, P. Andrade, F. A. Tomas-Barberan, *J. Agric. Food Chem.* **1996**, *44*, 2053.
[18] F. Bohlmann, P. K. Mahanta, J. Jakupovic, R. C. Rastogi, A. A. Natu, *Phytochemistry* **1978**, *17*, 1165.
[19] F. Bohlmann, P. K. Mahanta, *Phytochemistry* **1979**, *18*, 887.
[20] F. Wang, K. Yang, F. C. Ren, J. K. Liu, *Fitoterapia* **2009**, *80*, 21.
[21] C. A. L. Bercht, H. M. Samrah, R. J. J. C. Luosberg, H. Theuns, C. A. Salemink, *Phytochemistry* **1976**, *15*, 830.
[22] G. O. Lobitz, G. Tamayo-Castillo, I. Merfort, *Phytochemistry* **1997**, *46*, 161.
[23] H. Heymann, Y. Tezuka, T. Kikuchi, S. Supriyatna, *Chem. Pharm. Bull.* **1994**, *42*, 138.
[24] K. He, L. Zeng, G. Shi, G.-X. Zhao, J. F. Kozlowski, J. L. Mclaughlin, *J. Nat. Prod.* **1997**, *60*, 38.

Received January 26, 2010