New Guaipyridine Sesquiterpene Alkaloids from *Artemisia rupestris* L.

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Three new guaipyridine sesquiterpene alkaloids, rupestine A, B, C (**1**–**3**, resp.), and the new nor-sesquiterpne alkaloid rupestine D (**4**) were obtained from the flowers of *Artemisia rupestris* L. Their structures were elucidated on the basis of sepectroscopic data and by comparison with those of the related compounds reported in the literature. In addition, the absolute configurations of **2** and **4** were determined by single-crystal X-ray diffraction analyses.

Introduction. – *Artemisia rupestris* L. (*Compositae*) is a well-known traditional Chinese medicinal plant in Xinjiang Uyghur Autonomous Region of China used for detoxification, with antitumor, antibacterial, and antivirus properties, and is used as well for protecting the liver [1][2]. In the last 20 years, sesquiterpenes, flavonoids, and other constituents from this species have been reported [3–9]. A new guaipyridine sesquiterpene alkaloid named rupestine was isolated by high-speed countercurrent chromatography in our laboratory [10], and this type of compounds has been found rarely in nature in the course of investigation. As a continuing investigation of bioactive metabolites of the plant, three new guaipyridine sesquiterpene alkaloids, named rupestines A, B, C (**1**–**3**, resp.), and one new natural compound (**4**), named rupestine D, were isolated (*Fig. 1*). The present article describes the isolation and structural characterization of these compounds.

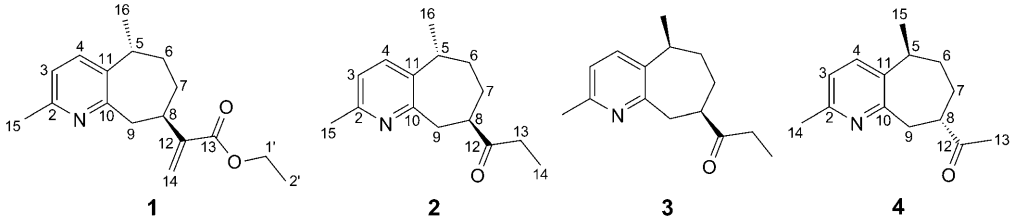

Fig. 1. *Compounds* **1**–**4** *isolated from Artemisia rupestris* L.

Results and Discussion. – Compound **1** (rupestine A), a light yellow oil, gave a positive *Dragendorff* test result and exhibited a molecular formula of $C_{17}H_{23}NO_2$ as

determined by ESI-MS (positive-ion mode: $[M+H]^+$ *m/z* 273.9) and ^{13}C-NMR, with seven degrees of unsaturation. The DEPT spectra indicated that compound **1** possesses five quaternary C-atoms and four CH, five CH_2, and three Me groups. The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) of **1** were quite similar to those of rupestine [10], which suggested that **1** is a guaipyridine sesquiterpene alkaloid and has the same basic skeleton as rupestine [10][11]. Compared with rupestine, a significant difference was that **1** showed characteristic signals for an EtO substituent (δ(H) 4.21 (*q*, *J* = 7.2) and

Table 1. *^{1}H-NMR Data of Compounds* **1**–**4** ($CDCl_3$, 400 MHz, δ in ppm, *J* in Hz)

	1	**2**	**3**	**4**
H–C(3)	6.92 (*d*, *J* = 8.0)	6.98 (*d*, *J* = 8)	6.94 (*d*, *J* = 7.6)	7.00 (*d*, *J* = 8.0)
H–C(4)	7.31 (*d*, *J* = 8.0)	7.38 (*d*, *J* = 8)	7.33 (*d*, *J* = 7.6)	7.40 (*d*, *J* = 8.0)
H–C(5)	3.04 (*m*)	2.99–3.04 (*m*)	2.95–3.04 (*m*)	2.94–3.04 (*m*)
H_a–C(6)	1.76–1.84 (*m*)	1.22–1.27 (*m*)	1.72–1.88 (*m*)	1.23–1.30 (*m*)
H_b–C(6)		1.82–1.94 (*m*)		1.82–1.94 (*m*)
H_a–C(7)	1.76–1.84 (*m*)	1.82–1.94 (*m*)	1.72–1.88 (*m*)	1.82–1.94 (*m*)
H_b–C(7)		1.98–2.08 (*m*)	2.00–2.10 (*m*)	2.04–2.11 (*m*)
H–C(8)	2.75–2.85 (*m*)	2.47–2.60 (*m*)	2.67–2.75 (*m*)	2.54–2.60 (*m*)
H_a–C(9)	3.15 (*d*, *J* = 13.6)	3.09 (*d*, *J* = 14)	3.16–3.24 (*m*)	3.13–3.28 (*m*)
H_b–C(9)	3.29 (*dd*, *J* = 14.4, 10)	3.24 (*dd*, *J* = 14, 10.8)	3.31–3.41 (*m*)	
CH_2(13) or Me(13)		2.52 (*q*, *J* = 7.2)	2.60 (*q*, *J* = 7.2)	2.23 (*s*)
H_a–C(14) or Me(14)	5.58 (*s*)	1.04 (*t*, *J* = 7.2)	1.02 (*q*, *J* = 7.2)	2.51 (*s*)
H_b–C(14)	6.18 (*s*)			
Me(15)	2.50 (*s*)	2.51 (*s*)	2.50 (*s*)	1.35 (*d*, *J* = 7.2)
Me(16)	1.30 (*d*, *J* = 7.2)	1.31 (*d*, *J* = 7.6)	1.31 (*d*, *J* = 7.6)	
CH_2(1′)	4.21 (*q*, *J* = 7.2)			
Me(2′)	1.31 (*t*, *J* = 7.2)			

Table 2. *^{13}C-NMR Data of* **1**–**5** ($CDCl_3$, 100 MHz, δ in ppm)

	1	**2**	**3**	**4**
C(2)	154.63	154.39	154.63	154.36
C(3)	121.04	121.17	121.42	121.24
C(4)	136.79	132.48	136.59	132.60
C(5)	37.94	34.79	37.65	34.76
C(6)	33.03	35.08	32.11	34.96
C(7)	31.68	33.22	28.40	32.92
C(8)	38.10	48.60	48.50	49.47
C(9)	43.78	39.73	39.57	39.43
C(10)	158.61	159.36	157.63	159.05
C(11)	137.61	137.90	138.14	137.90
C(12)	146.45	213.71	213.38	211.07
C(13)	167.04	34.38	34.29	28.49
C(14)	122.86	7.83	7.76	23.71
C(15)	23.86	23.79	23.56	20.32
C(16)	18.33	20.38	18.85	
C(1′)	60.66			
C(2′)	14.22			

1.31 (t, $J = 7.2$); δ(C) 60.66 and 14.22) at C(13), which could be confirmed by the HMBC correlations observed between H–C(1′) and the ester CO group. Unambiguous complete assignments for the ^{1}H- and ^{13}C-NMR signals of compound **1** were completed by the combined interpretation of DEPT, HMBC, and HSQC spectra (see *Tables 1* and *2*). Hence, the structure of **1** was determined to be as shown and named rupestine A. In the study, the plant was extracted by EtOH, so compound **1** could be an artefact of isolation, derived from rupestine.

Compound **2** (rupestine B), colorless crystals, gave a positive *Dragendorff* test result and exhibited a molecular formula of $C_{15}H_{21}NO$, as determined by ESI-MS (positive-ion mode: $[M+H]^+$ m/z 231.8), with six degrees of unsaturation. Compound **2** was recognized as a guaipyridine sesquiterpene alkaloid by ^{1}H- and ^{13}C-NMR data (see *Tables 1* and *2*), which were quite similar with those of rupestine, a significant difference was that the acryl group at C(8) was replaced by a propionyl moiety in **2** (δ(H) 2.52 (q, 2 H), 1.04 (t, 3 H); δ(C) 213.71 (C=O), 34.38 (CH_2), 7.83 (Me)), which could be confirmed by the key HMBC correlation of Me(14), CH_2(13), CH_2(9), and H–C(8) with C(12), of Me(14) with C(13), and of CH_2(13) with C(14). The absolute configuration was elucidated by single-crystal X-ray diffraction (*Fig. 2*). The structure of **2** was accordingly established and named rupestine B.

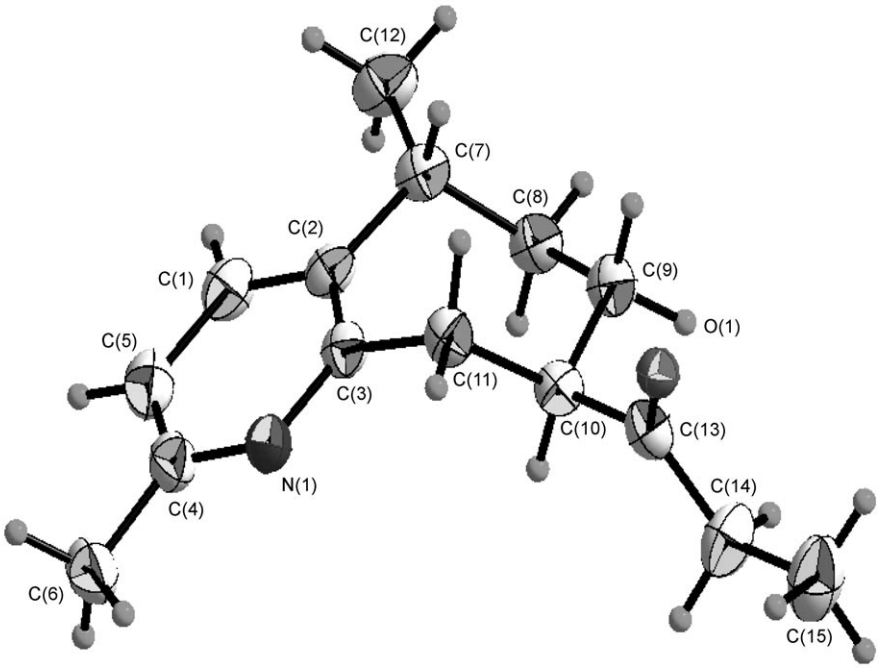

Fig. 2. *Crystallographic structure of rupestine B* (**2**)

Compound **3** (rupestine C), a light yellow oil, gave a positive *Dragendorff* test result, and exhibited a molecular formula of $C_{15}H_{21}NO$, as determined by ESI-MS (positive-ion mode: $[M+H]^+$ m/z 231.8), with six degrees of unsaturation. The NMR spectra of **3** were nearly identical to those of **2** (see *Tables 1* and *2*). After a careful

study of the DEPT, HMBC, and HSQC spectra, compound **3** was determined to be an epimer of **2**, and named rupestine C.

Compound **4** (rupestine D), white needles, gave a positive *Dragendorff* test result, and exhibited the molecular formula $C_{14}H_{19}NO$, as determined by HR-ESI-MS (positive-ion mode: $[M+H]^+$ *m/z:* 218.1535, calc. 218.1518), with six degrees of unsaturation. The ^{13}C-NMR and DEPT spectra showed 14 C-atoms, and the signals were similar to those of **2** and **3**. But in the ^{13}C-NMR spectrum, one methyl ketone signal was observed rather than an ethyl ketone as in **2** and **3**. Further configuration determination was conducted by analysis of the 2D-NMR spectra and single crystal X-ray diffraction (*Fig. 3*). Compound **4** was named rupestine D, a compound which has been synthesized before [12], but it was isolated from a natural source for the first time, and its NMR data assignment were completed in this article (*Tables 1* and *2*).

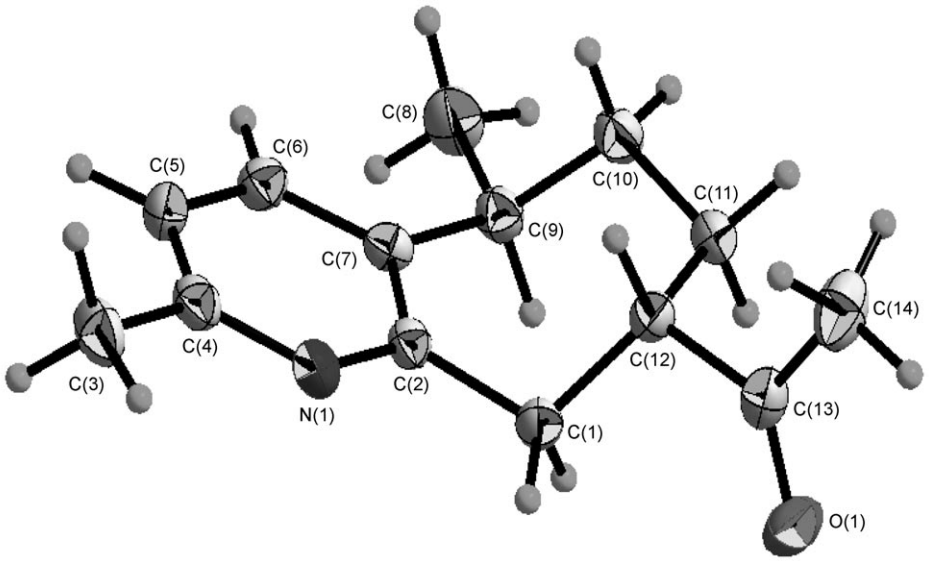

Fig. 3. *Crystallographic structure of rupestine D* (**4**)

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical, Ltd.*, Qingdao, P. R. China), *Sephadex LH-20* (*Pharmacia*), *Lichroprep RP-18* gel (*Merck*, Germany). $[\alpha]_D$: *PerkinElmer* Model *341LC* Polarimeter; IR Spectra (KBr): *EQUINOX-55* FT-IR spectrometer (*Bruker*, Germany), in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR spectra: *INOVA-400 NMR* (*Varian*, USA), at 295 K; δ in ppm rel. to Me_4Si, *J* in Hz. HR-FAB-MS: *AutoSpec Ultima-TOF* mass spectrometer (*Micromass*, UK); in *m/z*. ESI-MS: *Accu TOF CS* (*Jeol*, Japan).

Plant Material. The flowers of *A. rupestris* L. were collected from Buerjin County, Xinjiang Uyghur Autonomous Region, P. R. China, in June 2006, and were identified by Prof. *Shi-Ming Duan* (Xinjiang

Institute of Ecology and Geography, Chinese Academy of Sciences). A voucher specimen was deposited with the Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The air dried and powdered flowers of *A. rupestris* L. (15 kg) were extracted by percolation with 95% EtOH (10 × 40 l) at r.t. The extract was filtered, concentrated under reduced pressure and suspended in H_2O, and then adjusted to pH 2 with 2% HCl, and then extracted with $CHCl_3$. The aq. layer was treated with sat. NH_4OH to pH 10, then extracted with $CHCl_3$ to obtain the $CHCl_3$ soluble compounds (8.5 g). The $CHCl_3$ extracts were chromatographed over CC (SiO_2; petroleum ether (PE)/Me_2CO, 100 :0 to 30 :1), *Sephadex LH-20* ($CHCl_3$/MeOH 1 :1), and preparative HPLC (40 – 80% aq. MeOH) to yield rupestine A (**1**; 2 mg), rupestine B (**2**; 15 mg), rupestine C (**3**; 2 mg), and rupestine D (**4**; 30 mg).

Rupestine A (= *Ethyl 2-[(5*R,*8*R*)-6,7,8,9-Tetrahydro-2,5-dimethyl-5*H*-cyclohepta[*b*]pyridin-8-yl]-prop-2-enoate*; **1**). Light yellow oil. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 273.9 ($[M+H]^+$).

Rupestine B (=*1-((5*R,*8*R*)-6,7,8,9-Tetrahydro-2,5-dimethyl-5*H*-cyclohepta[*b*]pyridin-8-yl)propan-1-one*; **2**). Colorless block crystals. M.p. 83 – 85°. $[\alpha]_D^{20} = +43$ ($c = 0.50$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 231.8 ($[M+H]^+$).

Rupestine C (=*1-((5*S,*8*R*)-6,7,8,9-Tetrahydro-2,5-dimethyl-5*H*-cyclohepta[*b*]pyridin-8-yl)propan-1-one*; **3**). Light yellow oil. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 231.8 ($[M+H]^+$).

Rupestine D (=*1-((5*S,*8*S*)-6,7,8,9-Tetrahydro-2,5-dimethyl-5*H*-cyclohepta[*b*]pyridin-8-yl)ethanone*; **4**). White needle crystals. M.p. 92 – 94°. $[\alpha]_D^{20} = -118$ ($c = 2.00$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 217.8 ($[M+H]^+$). HR-ESI-MS: 218.1535 ($C_{14}H_{19}NO^+$; calc. 218.1518).

Crystallographic Data of **2**[1]). Formula $C_{15}H_{21}NO$; $M_r = 231.33$; crystal size: 0.62 × 0.32 × 0.22 mm; crystal system: monoclinic ; space group *P*2(1); unit-cell dimensions: $a = 4.9852(10)$, $b = 11.793(2)$, $c = 10.688(2)$ Å, $\alpha = 90.00$, $\beta = 101.49(3)$, $\gamma = 90.00°$, $V = 615.7(2)$ Å^3; $Z = 2$; $D_x = 1.172$ mg/m^3; $F(000) = 236$, $T = 153(2)$ K. Diffraction data of **2** were collected with an *Rigaku R-AXIS SPEDER* area-detector diffractometer, using graphite-monochromated MoK_α radiation ($\lambda = 0.71073$ Å) and the ω to 2θ scan mode. The total number of reflections measured was 2785, of which 2495 were used for the solution of the structure. Final indices: $R_f = 0.0371$, $R_w = 0.0972$. The structure was solved by direct methods using SHELXS-97 and refined by full-matrix least-squares on F^2 using SHELXL-97.

Crystallographic Data of **4**[2]). Formula $C_{14}H_{19}NO$; $M_r = 217.31$; crystal size: 0.77 × 0.39 × 0.12 mm; crystal system: monoclinic; space group *P*2(1); unit-cell dimensions: $a = 4.9408(10)$, $b = 11.407(2)$, $c = 11.927(2)$ Å, $\alpha = 90.00$, $\beta = 100.51(3)$, $\gamma = 90.00°$, $V = 660.9(2)$ Å^3; $Z = 2$; $D_x = 1.162$ mg/m^3; $F(000) = 252$, $T = 153(2)$ K. Diffraction data of **4** were collected with an *Rigaku R-AXIS SPEDER* area-detector diffractometer, using graphite-monochromated MoK_α radiation ($\lambda = 0.71073$ Å) and the ω to 2θ scan mode. The total number of reflections measured was 3012, of which 2517 were observed. Final indices: $R_f = 0.0476$, $R_w = 0.1049$. The structure was solved by direct methods using SHELXS-97 and refined by full-matrix least-squares on F^2 using SHELXL-97.

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[1]) CCDC-699570 contains the supplementary crystallographic data for **2**. This data can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif.

[2]) CCDC-725542 contains the supplementary crystallographic data for **4**. This data can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif.

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