Three New Diterpenoids from *Salvia przewalskii* MAXIM

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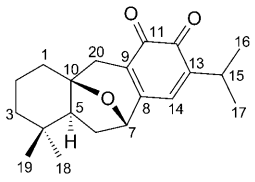
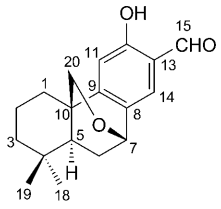
Three new compounds including one *ortho*-quinone-type icetexane diterpenoid, *i.e.*, przewalskin E (**1**), one abietane dinorditerpenoid, *i.e.*, przewalskin F (**2**), and one 7,20-epoxyabietane diterpenoid, *i.e.*, przewalskin G (**3**), were isolated from *Salvia przewalskii* MAXIM. The identification and structure elucidation of these compounds were based on 1D- and 2D-NMR spectral-data analysis. It is noteworthy that przewalskin E (**1**), featured with an 11,12-dioxoicetexane skeleton, represents the second example of a naturally occurring *ortho*-quinone-type icetexane diterpenoid from *Salvia* plants.

Introduction. – *Salvia przewalskii* MAXIM (Labiatae) is a plant endemic to northwest China and usually used as a surrogate of 'Tanshen' (*S. miltiorrihiza*) in Chinese folk medicine [1]. Many bioactive abietane diterpenoids, especially naphthoquinones, have been reported as the main secondary metabolites of this plant [2–4]. As a continuation of our systematic research work on the diterpenoids from *Salvia* plants [5–8], we examined the constituents of *S. przewalskii* MAXIM collected in Shangrila of Yunnan Province. In a previous article, we have reported two icetexane diterpenoids (przewalskins C and D) from this plant [7]. Further study of the chemical constituents of *S. przewalskii* MAXIM resulted in the isolation of three new diterpenoids, przewalskins E–G[1]) (**1**–**3**), of which przewalskin E (**1**), featuring an 11,12-dioxoicetexane skeleton, can be seen as the second example of a naturally occurring *ortho*-quinone-type icetexane diterpenoid from *Salvia* plants. The first *ortho*-quinone-type icetexane diterpenoid, romulogarzone, was isolated from *S. ballotaeflora* in 1976 [9].

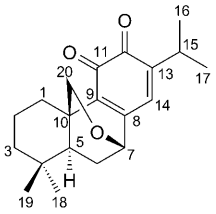
Results and Discussion. – Przewalskin E (**1**), obtained as a white powder, gave rise to a $[M+Na]^+$ peak at *m/z* 337.1778 in the positive-ion-mode HR-ESI-MS, corres-



1[1])



2[1])



3[1])

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

ponding to the molecular formula $C_{20}H_{26}O_3$. The IR spectrum of **1** exhibited the characteristic absorptions for the C=O groups of an *ortho*-quinone compound at 1722, 1680, and 1636 cm^{-1}. The NMR data (*Table*) indicated that **1** contained two conjugated C=O groups, four Me, five CH_2, and four CH groups (including an oxygenated and an olefinic one), as well as five quaternary C-atoms (including an oxygenated and three olefinic ones). Considering the structures of the compounds previously isolated from this genus and the formula of **1**, along with the characteristic CH signals at δ(C) 27.2 and 51.5 due to C(15) and C(5), respectively, a noticeable quaternary C-atom signal at δ(C) 32.0 assignable to C(4), and four characteristic Me signals at δ(C) 21.6 (C(16)), 21.5 (C(17)), 30.3 (C(18)), and 26.7 (C(19)), compound **1** could be ascribed to an icetexane or abietane diterpenoid [10–13]. Further analysis of the 1D-NMR spectra indicated that **1** was an icetexane diterpenoid, due to the absence of the signals of the characteristic quaternary C-atom C(10) (usually at δ(C) 38–42) and the Me(20) group (δ(C) 20–30) in the ^{13}C-NMR spectrum) of abietane diterpenoids [10–13]. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of 5,6-dihydrosalviasperanol (= *rel*-(4a*R*,10*R*,11a*R*)-1,2,3,4,5,10,11,11a-octahydro-1,1-dimethyl-8-(1-methylethyl)-4a,10-epoxy-4a*H*-dibenzo[*a*,*d*]cycloheptene-6,7-diol) indicated that they were strikingly similar, except for the presence of two conjugated C=O groups at δ(C) 180.3 and 179.7 in **1** which can be ascribed to C(11) and C(12), respectively, by the HMBCs CH_2(20)/C(11), H–C(14)/C(12), and H–C(15)/C(12) [11]. So, compound **1** was elucidated to be an *ortho*-quinone-type icetexane diterpenoid, namely the 11,12-dioxo derivative of 5,6-dihydrosalviasperanol, which was confirmed by ^{1}H,^{1}H-COSY and HMBC experiments. Analysis of the ^{1}H,^{1}H-COSY plot led to the identification of a $CH_2CH_2CH_2$ (C(1) to C(3)) and an i-Pr (C(15) to C(17)) fragment. The expected HMBCs (*Fig.*) H–C(5)/C(1), C(4), and C(6), H–C(7)/C(5), C(8), C(9), and C(14), CH_2(20)/C(5), C(7), C(9), and C(10), and H–C(14)/C(7), C(9), C(13), and C(15) were all found.

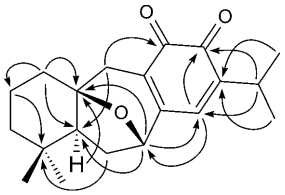


Figure. *Selected HMBC of przewalskin E* (**1**)

For biogenetic reasons, Me(19) of **1** is expected to be β-oriented, and H–C(5) and Me(18) α-oriented. This was confirmed by the NOE correlation Me(18)/H–C(5) found in the ROESY plot. Careful analysis of the molecular model by means of the HGS Molecular Structure Model B indicated that the distance between H–C(7) and Me(19) would allow a NOE correlation in case of an α-oriented H–C(7). If H–C(7) is β-oriented, the distance between Me(19) and H–C(20) is so short that an NOE Me(19)/H–C(20) is expected. In the ROESY plot, the NOE H–C(7)/Me(19) rather than Me(19)/H–C(20) was found, which indicated that H–C(7) was in the α-

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$, 400 and 100 MHz, resp.) *of Compounds* **1**, **2**, *and* **3**[1])[a]). δ in ppm, J in Hz.

	1		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
H_α–C(1)	29.8 (*t*)	1.91–1.94 (*m*)	28.5 (*t*)	1.76–1.86 (*m*)	28.8 (*t*)	2.36–2.46 (*m*)
H_β–C(1)		1.44–1.46 (*m*)		1.04–1.16 (*m*)		1.85–1.97 (*m*)
H_α–C(2)	15.8 (*t*)	1.72–1.78 (*m*)	18.6 (*t*)	1.50–1.77 (*m*)	18.6 (*t*)	1.56–1.64 (*m*)
H_β–C(2)		1.40–1.48 (*m*)				1.34–1.48 (*m*)
H_α–C(3)	31.6 (*t*)	1.54–1.66 (*m*)	40.9 (*t*)	1.50–1.77 (*m*)	40.6 (*t*)	1.40–1.59 (*m*)
H_β–C(3)		1.15–1.21 (*m*)				1.08–1.20 (*m*)
C(4)	32.0 (*s*)		34.0 (*s*)		33.8 (*s*)	
C(5)	51.5 (*d*)	1.18–1.28 (*m*)	42.0 (*d*)	1.16–1.28 (*m*)	42.2 (*d*)	1.28–1.36 (*m*)
H_α–C(6)	37.9 (*t*)	2.05–2.15 (*m*)	29.8 (*t*)	2.02–2.17 (*m*)	28.0 (*t*)	1.95–2.05 (*m*)
H_β–C(6)		1.58–1.72 (*m*)		1.52–1.68 (*m*)		1.58–1.76 (*m*)
H–C(7)	74.9 (*d*)	4.40 (*d*, J = 6.8)	69.5 (*t*)	4.76 (br. *s*)	70.1 (*d*)	4.46 (br. *s*)
C(8)	153.0 (*s*)		131.4 (*s*)		152.0 (*s*)	
C(9)	129.5 (*s*)		156.6 (*s*)		139.9 (*s*)	
C(10)	80.4 (*s*)		38.6 (*s*)		40.2 (*s*)	
H–C(11)	180.3 (*s*)		109.1 (*d*)	6.71 (*s*)	180.2 (*s*)	
C(12)	179.7 (*s*)		162.4 (*s*)		177.4 (*s*)	
C(13)	147.9 (*s*)		118.2 (*s*)		149.6 (*s*)	
CH(14)	131.9 (*d*)	6.45 (*s*)	127.0 (*d*)	7.15 (*s*)	132.3 (*d*)	6.60 (*s*)
CH(15)	27.2 (*d*)	2.92 (*sept.*, J = 6.8)	196.0 (*d*)	9.75 (*s*)	27.4 (*d*)	2.92 (*sept.*, J = 6.8)
Me(16)	21.6 (*q*)	1.09 (*d*, J = 6.8)			21.4 (*q*)	1.09 (*d*, J = 6.8)
Me(17)	21.5 (*q*)	1.09 (*d*, J = 6.8)			21.5 (*q*)	1.08 (*d*, J = 6.8)
Me(18)	30.3 (*q*)	0.95 (*s*)	32.7 (*q*)	0.75 (*s*)	33.1 (*q*)	0.85 (*s*)
Me(19)	26.7 (*q*)	0.83 (*s*)	20.9 (*q*)	1.07 (*s*)	20.8 (*q*)	1.07 (*s*)
CH_2(20)	38.0 (*t*)	2.46 (*d*, J = 18.3), 2.15 (*d*, J = 18.3)	67.1 (*t*)	4.25 (*d*, J = 11.0), 2.78 (*d*, J = 11.0)	67.7 (*t*)	4.26 (*d*, J = 9.6), 3.00 (*d*, J = 9.6)

[a]) Assignments were confirmed by ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments.

orientation. So, the structure of **1** was elucidated as (7β)-7,10-epoxyicetexa-8,13-diene-11,12-dione[1]), and named przewalskin E.

Przewalskin F (**2**), obtained as a white powder, gave a molecular-ion peak at m/z 286 in the EI-MS, consistent with the molecular formula $C_{18}H_{28}O_3$ determined by the positive-ion-mode HR-ESI-MS (m/z 309.1467 $[M+Na]^+$). This suggested that compound **2** is a dinorditerpenoid. This deduction was confirmed by the ^{13}C-NMR spectrum, which exhibited signals for 18 C-atoms. On the basis of careful analysis of the ^{1}H- and ^{13}C-NMR data (*Table*) and 2D-NMR spectra, przewalskin F (**2**) was identified as (7β)-12-hydroxy-7,20-epoxy-16,17-dinorabieta-8,11,13-trien-15-al[1]). In the ^{13}C-NMR spectrum of **2**, the signals of six quaternary C-atoms (including four olefinic ones), five CH groups (including an aldehyde function, an oxygenated, and two olefinic C-atoms), five CH_2 moieties (including an oxygenated one), and two Me groups were present. Side-by-side comparison of the NMR data of **2** with those of przewalskin showed a good overall similarity, except that **2** contained an aldehyde function which was not present in przewalskin (=(4a*R*,9*S*,10a*S*)-1,3,4,9,10,10a-hexahydro-1,1,7-trimethy-2*H*-9,4a-(epoxymethano)phenanthren-6-ol) [4]. This aldehyde group was assigned to

C(15) by the HMBCs observed between the aldehyde H-atom at δ(H) 9.75 (*s*, 1 H) and C(11), C(12), C(13), and C(14). The relative configurations of C(5) and C(10) are assumed to be the same as those of przewalskin based on biogenetic reasons and confirmed by the NOEs Me(18)/H–C(5) and Me(19)/H_a–C(20) (δ(H) 4.25 (*d*, J = 11.0 Hz)) in the ROESY plot. The α-orientation of H–C(7) was deduced by the connection of C(7) and C(20) through an epoxy group.

The molecular formula of przewalskin G (**3**) was determined to be $C_{20}H_{26}O_3$ (eight degrees of unsaturation) by analysis of the NMR spectra, EI-MS, and positive-ion-mode HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra (*Table*) closely matched with those of carnosol (=(4a*R*,9*S*,10a*S*)-1,3,4,9,10,10a-hexahydro-5,6-dihydroxy-1,1-dimethyl-7-(1-methylethyl)-2*H*-9,4a-(epoxymethano)phenanthren-12-one), a known abietane diterpenoid previously obtained from the same genus [14]. A significant difference was the presence of two conjugated C=O groups (δ(C) 180.2 and 177.4) in **3** instead of two oxygenated aromatic C-atoms in carnosol. This observation strongly indicated that C(11) and C(12) of **3** were two conjugated C=O groups, which was confirmed by the HMBCs H–C(14)/C(12), H–C(14)/C(7), and H–C(15)/C(12). As in the case of **2**, for biogenetic reasons, Me(19) and C(20) of **3** were both assumed to be β-oriented, and H–C(5) and Me(18) α-oriented. This was confirmed by the NOEs Me(18)/H–C(5) and Me(19)/H_a–C(20) (δ(H) 4.26 (*d*, J = 9.6)). Then, H–C(7) was deduced to be in the α-orientation based on the preasence of the 7,20-epoxy group. Accordingly, the structure of **3** was elucidated as 7,20-epoxyabieta-8,13-diene-11,12-dione[1]), and given the trivial name przewalskin G.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh, 10–40 μm; *Qingdao Marine Chemical Inc.*), *Lichroprep RP-18* (43–63 μm; *Merck*), and *Sephadex LH-20* (*Pharmacia*). TLC: SiO_2 plates; visualization by spraying with 10% H_2SO_4 in EtOH, followed by heating. UV/VIS Spectra: *UV-2401-PC* spectrophotometer; λ_{max} (log ε) in nm. Optical rotations: *Horiba-SEPA-300* polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrometer; KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-400* spectrometer; at 400 and 100 MHz, resp.; δ in ppm, J in Hz. 2D-NMR Spectra: *Bruker DRX-500* NMR instrument. EI-MS: *VG-Auto-Spec-3000* spectrometer; in *m*/*z* (rel. %). ESI- and HR-ESI-MS: *API-Qstar-Pulsar* instrument.

Plant Material. Plants of *Salvia przewalskii* (whole plant) were collected in Shangrila of Yunnan Province, in August 2002, and were identified by Prof. *Xi-Wen Li*, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 200216) was deposited with the Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The dried and powdered (11.9 kg) *S. przewalskii* were extracted with Me_2CO at r.t. (3 × 40 l). The solvent was evaporated and the gummy residue (310 g) subjected to CC (*DM-130* porous resin, MeOH/H_2O 5:5 and 9:1). The residue of the MeOH/H_2O 9:1 fraction was partitioned between H_2O and AcOEt. The AcOEt part was subjected to CC (SiO_2, petroleum ether/Me_2CO of increasing polarity): *Fractions 1–6* (by TLC). Compounds **1** (5 mg), **2** (6 mg), and **3** (5 mg) were all isolated from *Fr. 3* after repeated CC (SiO_2, $CHCl_3$/AcOEt 9:1; *Lichroprep RP-18*, MeOH/H_2O 7:3 → 1:0; *Sephadex LH-20*, $CHCl_3$/MeOH 1:1).

Przewalskin E (= rel-*(4a*R,*10*R,*11a*R*)-1,2,3,4,5,10,11,11a-Octahydro-1,1-dimethyl-8-(1-methylethyl)-4a,10-epoxy-4a*H-*dibenzo[*a,d*]cycloheptene-6,7-dione*; **1**): White powder. $[\alpha]_D^{22.4} = -64.9$ (c = 0.23,

$CHCl_3$). UV ($CHCl_3$): 239.6 (1.59), 272.4 (1.65). IR: 2926, 1722, 1680, 1636, 1445, 1366, 1338, 1243, 1170, 1045. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 337 ($[M+Na]^+$), 651 ($[2\,M+Na]^+$). HR-ESI-MS (pos.): 337.1778 ($[M+Na]^+$, $C_{20}H_{26}NaO_3^+$; calc. 337.1779).

Przewalskin F (= rel-*(4a*R*,9*S*,10a*S*)-1,3,4,9,10,10a-Hexahydro-6-hydroxy-1,1-dimethyl-2*H*-9,4a-(epoxymethane)phenanthrene-7-carboxaldehyde*; **2**): White powder. $[\alpha]_D^{19.3} = -54.4$ ($c = 0.16$, $CHCl_3$). UV ($CHCl_3$): 224.4 (1.32), 268.6 (1.64), 331.8 (1.09). IR: 3433, 2928, 1652, 1578, 1460, 1374, 1330, 1300, 1239, 1209, 1154, 1034, 941. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 286 (9, M^+), 257 (20), 256 (100), 218 (8), 189 (29), 185 (36), 157 (12), 128 (14), 115 (13), 83 (23), 72 (26). HR-ESI-MS (pos.): 309.1467 ($[M+Na]^+$, $C_{18}H_{22}NaO_3^+$; calc. 309.1466).

Przewalskin G (= rel-*(4a*R*,9*S*,10a*S*)-1,3,4,9,10,10a-Hexahydro-1,1-dimethyl-7-(1-methylethyl)-2*H*-9,4a-(epoxymethano)phenanthrene-5,6-dione*; **3**): White powder. $[\alpha]_D^{19.3} = +43.8$ ($c = 0.15$, $CHCl_3$). UV ($CHCl_3$): 240.24 (1.01). IR: 2956, 293, 2869, 1712, 1379, 1653, 1462, 1390, 1365, 1313, 1293, 1238, 1161, 1045, 913, 906. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 314 (6, M^+), 286 (100), 271 (15), 256 (22), 241 (54), 215 (13). HR-ESI-MS (pos.): 337.1785 ($[M+Na]^+$, $C_{20}H_{26}NaO_3^+$; calc. 337.1779).

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