Chem. Pharm. Bull. **38**(4) 942—944 (1990) Vol. 38, No. 4

New Triterpenes from a Chinese Medicine, Goreishi

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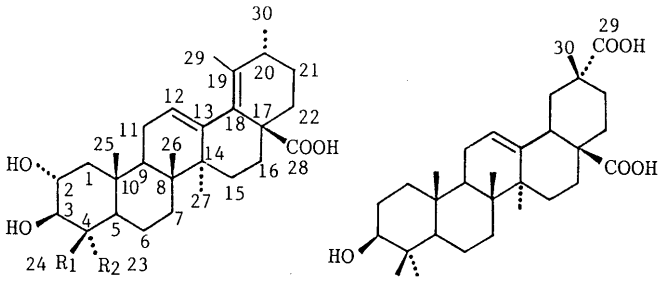
Besides serratagenic acid, three new ursane-type triterpenes, named goreishic acids I (1), II (2), and III (3), were isolated from a Chinese medicine, Goreishi (the feces of *Trogopterus xanthipes* MILNE-EDWARDS). The structures of 1, 2 and 3 were respectively determined as 2α,3β-dihydroxyursa-12,18-dien-28-oic acid, 2α,3β-dihydroxy-23-norursa-12,18-dien-28-oic acid and 2α,3β-dihydroxy-24-norursa-12,18-dien-28-oic acid on the basis of spectroscopic evidence.

Keywords Goreishi; *Trogopterus xanthipes*; goreishic acid I; goreishic acid II; goreishic acid III; serratagenic acid; Chinese medicine; triterpene; 23-norursane type; 24-norursane type

Previously,[1] we reported the isolation and characterization of triterpenes having cytotoxicity against P-388 lymphocytic leukemia cells from a Chinese traditional medicine, Goreishi (the feces of *Trogopterus xanthipes* MILNE-EDWARDS), which had exhibited tumor-inhibitory activity in the Ehrlich ascites carcinoma *in vivo* bioassay.[2] A continuing search for cytotoxic compounds from this medicine has led to the isolation of three new and one known triterpenes, which exhibited no significant cytotoxicity against the P-388 culture cells. We report here the structural elucidation of these triterpenes.

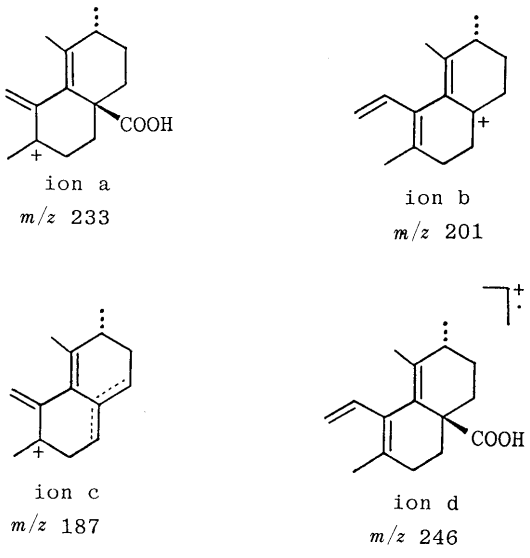
The $CHCl_3$ fraction was obtained by a solvent partitioning sequence from the 80% MeOH extract of Goreishi as described previously.[1] The fraction was separated by Sephadex LH-20 and repeated silica gel column chromatographies and high-performance liquid chromatography (HPLC) to afford three new compounds named goreishic acids I (**1**), II (**2**) and III (**3**), besides serratagenic acid (**4**).



1 : $R_1=R_2=CH_3$
2 : $R_1=CH_3$, $R_2=H$
3 : $R_1=H$, $R_2=CH_3$

4

Chart 1



ion a *m/z* 233

ion b *m/z* 201

ion c *m/z* 187

ion d *m/z* 246

Chart 2

Serratagenic acid (**4**) was identified by comparison of the physical constants and the proton and carbon-13 nuclear magnetic resonance (^1H- and ^{13}C-NMR) spectra with reference data.[3] This compound has been isolated as an aglycone of glycosides from *Nothopanax delavayi*[3] and *Clerodendron serratum*.[4] This is the first isolation of the free acid.

The molecular formula of goreishic acid I (**1**), mp 189—199 °C (MeOH), $[\alpha]_D^{26}$ +161° (c=0.6, MeOH), was determined as $C_{30}H_{46}O_4$ on the basis of the high-resolution mass spectrum (HR-MS) [*m/z*: 470.3385 (M^+)]. In the electron ionization (EI)-MS, **1** exhibited significant fragment peaks at *m/z*: 233, 201, 187 and 246 ascribable to the ions a, b, c and d, respectively, which resulted from cleavage of ring C under electron impact.[5] The molecular formulae of these ions were supported by the HR-MS. In addition to this evidence, the observation of one *sec*-methyl and six *tert*-methyl signals in the ^1H-NMR spectrum (Table I) of this compound suggested that **1** was a Δ^{12}-unsaturated triterpene. The appearance of four carbon signals of double bonds in the ^{13}C-NMR spectrum (Table I) and the absorption band of a heteroannular diene at 227 nm in the ultraviolet (UV) spectrum revealed that the extra double bond was placed between C-18 and C-19. The ^1H-NMR spectrum of **1** exhibited an olefinic proton signal at δ 5.72 and an allylic methyl proton signal at δ 1.86. Since the olefinic proton signal is due to the 12-proton as deduced from coupling with the 11-protons and analysis of the X-nucleus-proton correlation with fixed evolution time (XCORFE) 2D-NMR spectrum (Table II), the allylic methyl group should be located at the 19-position. This assignment was supported by the fact that the 19-methyl proton signal correlated with the 18- or 19-carbon signal in the XCORFE spectrum of **1** (Table II). Since this evidence shows **1** to be an ursane-type triterpene, the methyl proton signal observed as a doublet at δ 1.12 in the ^1H-NMR spectrum is assigned to the 20-methyl protons. The coupling constant of 12.8 Hz between the 20- and 21α-protons shows the 20-methyl group to be affixed in an α-equatorial manner. The infrared (IR), and ^1H- and ^{13}C-NMR spectra indicated that **1** possessed three functionalities, namely, two hydroxyl groups and a carboxylic acid unit. Occurrence of

TABLE I. ^{1}H- and ^{13}C-NMR Chemical Shifts (δ ppm) and Coupling Constants (J_{H-H} Hz, in Parentheses) of **1**, **2** and **3** in Pyridine-d_5

Position		Proton 1		Proton 2		Proton 3		Carbon 1	Carbon 2	Carbon 3
1	α	1.33 t	(12.5)	1.28 t	(12.5)	1.38 dd	(12.8, 11.7)	48.45	48.87	47.94
	β	2.35 dd	(12.5, 4.2)	2.32 dd	(12.5, 4.3)	2.38 dd	(12.8, 4.9)			
2		4.14 ddd	(12.5, 9.7, 4.2)	4.21 ddd	(12.5, 9.2, 4.3)	4.13 ddd	(11.7, 8.8, 4.9)	68.70	68.25	71.86
3		3.43 d	(9.7)	3.94 dd	(9.2, 5.6)	3.35 t	(8.8)	83.80	78.90	82.30
4				2.31 m		1.68 m		39.44	41.64	37.51
5								56.08	49.43	52.15
6								18.83	24.03	20.99
7								35.09	34.46	34.23
8								39.90	39.54	39.20
9		1.66 dd	(11.5, 5.2)	1.61 dd	(11.0, 5.0)	1.65 dd	(11.3, 5.4)	48.36	47.74	46.05
10								38.39	38.36	37.85
11	α	2.10 dt	(18.0, 5.2)	2.05 dt	(18.3, 5.0)	2.16 dt	(18.0, 5.4)	23.59	23.51	24.03
	β	1.98 ddd	(18.0, 11.5, 2.6)	1.96 ddd	(18.3, 11.0, 2.6)	1.98 ddd	(18.0, 11.3, 2.0)			
12		5.72 dd	(5.2, 2.6)	5.71 dd	(5.0, 2.6)	5.73 dd	(5.4, 2.0)	125.87	125.84	126.13
13								139.50	139.52	139.69
14								45.09	45.10	45.16
15								29.16	29.13	29.04
16		1.39 m		1.44 m		1.40 m		27.06	27.05	27.06
		2.25 m		2.22 m		2.23 m				
17								49.79	49.79	49.79
18								134.65[b] a,b)	134.65[b] a,b)	134.66[b] a,b)
19										
20		2.28 m		2.24 m		2.26 m		34.84	34.83	34.84
21	α	1.76 qd	(12.8, 3.8)	1.76 qd	(12.8, 3.0)	1.73 qd	(12.7, 3.0)	31.90	31.89	31.90
	β	2.19 br d	(12.8)	2.21 br d	(12.8)	2.21 br d	(12.7)			
22	α	2.59 dt	(12.8, 3.8)	2.58 dt	(13.0, 3.0)	2.59 dt	(12.7, 3.0)	35.61	35.58	35.62
	β	1.54 td	(12.8, 3.8)	1.53 td	(13.0, 3.0)	1.55 td	(12.7, 3.0)			
23		1.29 s				1.30 d	(6.3)	29.42	—	15.94
24		1.10 s		1.22 d	(7.6)			17.81	11.10	—
25		1.03 s		1.00 s		0.95 s		17.65	18.09	15.94
26		1.07 s		1.04 s		1.07 s		18.38	18.35	18.46
27		1.14 s		1.12 s		1.14 s		22.14	22.22	22.06
28								178.66	178.69	178.65
29		1.86 s		1.85 s		1.85 s		19.69	19.67	19.67
30		1.12 d	(8.8)	1.16 d	(7.0)	1.12 d	(7.0)	18.93	18.94	18.94

a) Signals were not detected due to overlap with the carbon signals of pyridine-d_5. The signals appeared at δ *ca.* 137.4 ppm in MeOH-d_4. *b*) Assignments may be reversed in each column.

TABLE II. Correlated Peaks in the XCORFE Spectra of **1** and **2**

1		2	
C (position)	H (position)	C (position)	H (position)
		1	25
		2	4
		3	1
4	23, 24	4	24
		7	26
9	25		
12	11		
13	27	13	27
14	12		
		15	27
17	16		
18 or 19	29	18 or 19	29
20	22		
23	24		
24	23		

the 17-carbon signal at δ 49.79 and the 22α-proton signal at lower field (δ 2.59) than observed for the other methylene proton signals shows the carboxylic acid moiety to be located at the 17-position. The two *sec*-hydroxyl-bearing methine proton signals at δ 4.14 (ddd, J=12.5, 9.7, 4.2 Hz) and δ 3.43 (d, J=9.7 Hz), and the methylene proton signals at δ 1.33 (t, J=12.5 Hz) and δ 2.35 (dd, J=12.5, 4.2 Hz) in the ^{1}H-NMR spectrum were respectively assigned to the 2β-, 3α-, 1α- and 1β-protons on the basis of the splitting pattern and coupling constants. Based on this evidence, **1** was assigned as 2α,3β-dihydroxyursa-12,18-dien-28-oic acid. Though the mixture of the diacetate of methyl ester of this acid and its isomer has been prepared from methyl diacetyltormentate by treatment with $SOCl_2$ and pyridine,[6] this is the first isolation from natural sources. Incidentally, assignments of the ^{1}H- and ^{13}C-NMR spectra of **1** as well as the other compounds listed in Table I were based on the application of ^{1}H–^{1}H decoupling, distortionless enhancement by polarization transfer (DEPT), and ^{1}H–^{13}C heteronuclear shift-correlated (HETCOR) and XCORFE (Table II) 2D-NMR spectra, and comparison with the published data for tormentic acid.[1]

The molecular formula of goreishic acid II (**2**), mp 174—175 °C (MeOH), $[\alpha]_D^{26}$ +203° (c=0.6, MeOH), was determined as $C_{29}H_{44}O_4$ on the basis of HR-MS [m/z: 456.3249 (M^+)]. As observed in **1**, **2** exhibited significant fragment ions a, b, c and d arising from cleavage of ring C in the EI-MS and the absorption band of a heteroannular diene at 228 nm in the UV spectrum. The ^{1}H and ^{13}C-NMR spectra revealed the presence of two hydroxyl groups, one carboxylic acid unit, two *sec*-methyl and three *tert*-methyl

groups besides one allylic methyl group. The ^{13}C-NMR chemical shifts of nineteen carbons in **2** were almost identical with those of C-8, 10—22 and 26—30 of **1**. Therefore, the rings C, D and E of **2** were expected to have the same structure as those of **1**. The two *sec*-hydroxyl-bearing methine proton signals at δ 4.21 (ddd, J=12.5, 9.2, 4.3 Hz) and 3.94 (dd, J=9.2, 5.6 Hz), and the methylene proton signals at δ 1.28 (t, J=12.5 Hz) and 2.32 (dd, J=12.5, 4.3 Hz) in the ^{1}H-NMR spectrum of **2** were respectively assigned to the 2β-, 3α-, 1α- and 1β-protons on the basis of the splitting pattern and coupling constants. The splitting pattern of the 3-proton signal in the ^{1}H-NMR spectrum suggested the presence of one proton at the 4-position. Occurrence of a cross peak with the 2-carbon signal in the XCORFE spectrum served to detect the 4-proton (δ 2.31), though the number was not clarified due to overlap with other protons. However, it was deduced from the DEPT spectrum that the 4-carbon assigned by correlation with the 4-proton signal in the HETCOR spectrum had one proton. In the XCORFE spectrum, the 4-carbon signal had a cross peak with the methyl proton signal of the doublet (J=7.6 Hz) at δ 1.22, showing the presence of a methyl group at the 4-position. The coupling constant of 5.6 Hz between the 3- and 4-protons suggested that the proton and methyl group at the 4-position were affixed in α-equatorial and β-axial manners, respectively. Based on this evidence, the structure of **2** was established as 2α,3β-dihydroxy-23-norursa-12,18-dien-28-oic acid.

Goreishic acid III (**3**), mp 188—190 °C (MeOH), $[\alpha]_D^{26}$ +266° (c=0.5, MeOH), has the same molecular formula as **2**, as deduced from the HR-MS. The general spectral features of this compound closely resembled those of **2** except for the chemical shifts of the 2- and 3-position signals and the coupling constant of the 3-proton signal in the ^{1}H-NMR spectrum. The coupling constant of 8.8 Hz between the 3- and 4-protons suggested that the proton and methyl group at the 4-position should be affixed in β-axial and α-equatorial manners, respectively. Based on this evidence, **3** was assigned as 2α,3β-dihydroxy-24-norursa-12,18-dien-28-oic acid.

The triterpenes **1**—**4** were tested for activity against P-388 cells in culture and exhibited ED_{50} values of 17.5, 23, 31 and 84 μg/ml, respectively, which are regarded as representing insignificant activity.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The UV spectra were recorded with a Hitachi 323 spectrophotometer and the IR spectra with a Perkin Elmer 1720 X FT-IR spectrometer. Optical rotations were measured on a JASCO ORD/UV-5 spectropolarimeter. The NMR spectra were recorded at 27 °C on a Varian XL-300 spectrometer, operating at 300 and 75.4 MHz for ^{1}H and ^{13}C, respectively, in pyridine-d_5 with tetramethylsilane (TMS) as an internal reference. The same parameters as described previously[1] were used for the 2D experiments. The EI- and HR-MS were taken on a Hitachi M 80 spectrometer. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and a Shim-pack PREP-ODS column (25 cm × 20 mm i.d.).

Extraction and Separation Goreishi (3 kg) were extracted with 80% MeOH, and the $CHCl_3$ fraction (37.9 g), obtained by a solvent partitioning sequence from the MeOH extract, was passed through Sephadex LH-20 using $CHCl_3$–MeOH (1 : 1) as described previously.[1] The fourth fraction (10.2 g) was chromatographed on a silica gel column with an MeOH–$CHCl_3$ gradient as the eluent. The fraction (3.15 g) eluted with 5% MeOH in $CHCl_3$ was repeatedly chromatographed on a silica gel column with an MeOH–$CHCl_3$ gradient to afford a crystalline substance (491 mg), which was finally subjected to HPLC with 90% MeOH, giving **1** (9 mg), **2** (13 mg), **3** (6 mg) and **4** (10 mg).

Goreishic Acid I (1) Colorless needles, mp 198—199 °C (MeOH), $[\alpha]_D^{26}$ +161° (c=0.6, MeOH). EI-MS m/z (%): 470 (43), 424 (16), 246 (10), 233 (100), 201 (66), 187 (58). HR-MS m/z: 470.3385 (M^+) (Calcd for $C_{30}H_{46}O_4$: 470.3393), 424.3346 ($M^+ - HCO_2H$) (Calcd for $C_{29}H_{44}O_2$: 424.3339), 246.1628 (ion d) (Calcd for $C_{16}H_{22}O_2$: 246.1619), 233.1536 (ion a) (Calcd for $C_{15}H_{21}O_2$: 233.1540), 201.1643 (ion b) (Calcd for $C_{15}H_{21}$: 201.1642), 187.1470 (ion c) (Calcd for $C_{14}H_{19}$: 187.1485). UV λ_{max}^{MeOH} nm (log ε): 227 (3.77). IR ν_{max}^{KBr} cm^{-1}: 3600—2400, 1694 (CO_2H), 1640 (C=C). The ^{1}H- and ^{13}C-NMR data are given in Table I.

Goreishic Acid II (2) Colorless plates, mp 174—175 °C (MeOH), $[\alpha]_D^{26}$ +203° (c=0.6, MeOH). EI-MS m/z (%): 456 (43), 410 (32), 246 (33), 233 (100), 201 (53), 187 (59). HR-MS m/z: 456.3249 (M^+) (Calcd for $C_{29}H_{44}O_4$: 456.3236), 410.3178 ($M^+ - HCO_2H$) (Calcd for $C_{28}H_{42}O_2$: 410.3182), 246.1624 (ion d) (Calcd for $C_{16}H_{22}O_2$: 246.1619), 233.1544 (ion a) (Calcd for $C_{15}H_{21}O_2$: 233.1540), 201.1645 (ion b) (Calcd for $C_{15}H_{21}$: 201.1642), 187.1484 (ion c) (Calcd for $C_{14}H_{19}$: 187.1485). UV λ_{max}^{MeOH} nm (log ε): 228 (3.73). IR ν_{max}^{KBr} cm^{-1}: 3600—2400, 1696 (CO_2H), 1650 (C=C). The ^{1}H- and ^{13}C-NMR data are given in Table I.

Goreishic Acid III (3) Colorless plates, mp 188—190 °C (MeOH), $[\alpha]_D^{26}$ +266° (c=0.5, MeOH). EI-MS m/z (%): 456 (26), 410 (14), 246 (17), 233 (100), 201 (54), 187 (58). HR-MS m/z: 456.3234 (M^+) (Calcd for $C_{29}H_{44}O_4$: 456.3236), 410.3160 ($M^+ - HCO_2H$) (Calcd for $C_{28}H_{42}O_2$: 410.3182), 246.1630 (ion d) (Calcd for $C_{16}H_{22}O_2$: 246.1619), 233.1531 (ion a) (Calcd for $C_{15}H_{21}O_2$: 233.1540), 201.1640 (ion b) (Calcd for $C_{15}H_{21}$: 201.1642), 187.1470 (ion c) (Calcd for $C_{14}H_{19}$: 187.1485). UV λ_{max}^{MeOH} nm (log ε): 229 (3.93). IR ν_{max}^{KBr} cm^{-1}: 3600—2400, 1692 (CO_2H), 1650 (C=C). The ^{1}H- and ^{13}C-NMR data are given in Table I.

Serratagenic Acid (4) Colorless needles, mp 299—300 °C (MeOH), $[\alpha]_D^{26}$ +38.77° (c=0.2, MeOH). EI-MS m/z (%): 486 (3), 440 (1), 278 (100), 233 (22). HR-MS m/z (%): 486.3339 (M^+) (Calcd for $C_{30}H_{46}O_5$: 486.3342), 440.3283 ($M^+ - HCO_2H$) (Calcd for $C_{29}H_{44}O_3$: 440.3287), 278.1522 [*retro*-Diels–Alder (RDA) ion] (Calcd for $C_{16}H_{22}O_4$: 278.1516), 233.1539 (RDA ion $-CO_2H$) (Calcd for $C_{15}H_{21}O_2$: 233.1540). IR ν_{max}^{KBr} cm^{-1}: 3600—2400, 1702 (CO_2H), 1640 (C=C). ^{1}H-NMR (pyridine-d_5) δ ppm: 0.88 (1H, t, J=11.0 Hz, 5-H), 0.91 (3H, s, 25-H), 1.04 (3H, s, 24-H), 1.05 (3H, s, 26-H), 1.24 (3H, s, 23-H), 1.30 (3H, s, 27-H), 1.60 (3H, s, 30-H), 1.69 (1H, dd, J=10.0, 4.0 Hz, 9-H), 2.04 (1H, dd, J=13.5, 3.5 Hz, 19β-H), 2.63 (1H, t, J=13.5 Hz, 19α-H), 3.45 (1H, dd, J=9.0, 6.0 Hz, 3α-H), 3.47 (1H, dd, J=13.5, 3.5 Hz, 18β-H), 5.58 (1H, m, 12-H). ^{13}C-NMR (pyridine-d_5) δ ppm: 15.57 (C-25), 16.58 (C-24), 17.45 (C-26), 18.80 (C-6), 20.04 (C-30), 23.86 (C-11, 16), 26.10 (C-27), 28.13 (C-2), 28.31 (C-15), 28.77 (C-23), 29.28 (C-21 or 22), 32.39 (C-21 or 22), 33.25 (C-7), 37.41 (C-10), 38.94 (C-1), 39.40 (C-4), 39.79 (C-8), 41.11 (C-18), 41.11 (C-19), 42.19 (C-20), 42.57 (C-14), 46.65 (C-17), 48.10 (C-9), 55.78 (C-5), 78.06 (C-3), 123.59 (C-12), 179.95 (C-28 or 29), 181.0 (C-28 or 29).

Acknowledgement We are very grateful to Miss M. Danjo of this university for the EI-MS measurements.

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