A Novel Atisane Diterpenoid from *Spiraea japonica* var. *acuta*

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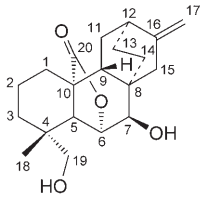
A novel *ent*-atisane lactone, spiramilactone E (**1**), was isolated from *Spiraea japonica* var. *acuta* YU. Its structure was elucidated by extensive spectroscopic analyses, and unequivocally confirmed by single-crystal X-ray diffraction (*Fig. 2*). Compound **1** contains a γ-lactone moiety between the 6-OH function and C(20), and β-configuration for the 7-OH group, in contrast to known related diterpenes previously isolated from the *S. japonica* complex.

1. Introduction. – The species *Spiraea japonica* L. (Rosaceae) contains seven varieties that are widely distributed in Yunnan Province, P. R. China. The young leaves, fruits, and roots of these plants have been used as diuretic, detoxicant, and analgesic agents, as well as for the treatment of inflammation, cough, headache, and toothache in traditional Chinese medicine (TCM) [1].

Our group systematically studied the chemical constituents of the complex *S. japonica*, and isolated 22 new hetisine-type alkaloids, 35 new atisine-type alkaloids, and seven new atisane-type diterpenoids [2][3], some of which were shown to exhibit significant bioactivity, including anti-inflammation, anti-platelet aggregation, and neuroprotective effects. Spiramine Q, for example, an atisine-type diterpene alkaloid from *S. japonica* var. *incise* was shown *in vitro* and *ex vivo* to decrease mouse mortality caused by intravenous injection of arachidonic acid, being more active than *Aspirin*® [4][5]. Spiramine T, an atisine-type diterpene alkaloids from *S. japonica* var. *acuta*, exhibited marked neuroprotective effects on the cerebral ischemia-reperfusion injury in gerbils [6][7].

In a search for additional diterpenes from this plant complex, a novel atisane-type diterpene, spiramilactone E (**1**), was isolated from the extract of *S. japonica* var. *acuta* YU. In this paper, we describe the isolation and structural elucidation of this new lactone.



Spiramilactone E (**1**)

2. Results and Discussion. – The roots of *S. japonica* var. *acuta* were extracted with 95% EtOH. The neutral part of the EtOH extract was purified by successive column chromatography on silica gel and *Sephadex LH-20* to afford spiramilactone E (**1**).

Compound **1**, obtained as a colorless, lumpish crystal, showed the $[M+Na]^+$ peak at *m/z* 355.1888 by HR-ESI-MS, in accord with the molecular formula $C_{20}H_{28}O_4$. The IR spectrum showed the presence of OH (3414 cm^{-1}), C=O (1754 cm^{-1}), and C=C (1645 cm^{-1}) functions. The ^{1}H-NMR spectrum of **1** (*Table*) showed signals due to a Me group at a quaternary C-atom (δ(H) 1.32 (*s*)), an oxygenated CH_2 (δ(H) 3.72, 3.97 (2*d*)), an exocyclic methylidene group (δ(H) 4.86, 4.95 (2*d*)), and two oxygenated CH (δ(H) 3.98 (br. *s*), 5.04 (*d*)).

Table. *^{1}H- and ^{13}C-NMR Data* of **1**. At 400/100 MHz, resp., in C_5D_5N; δ in ppm, *J* in Hz.

Atom	δ(H)	δ(C)	^{1}H,^{1}H-COSY[a])	HMBC (H → C)[b])
CH_2(1)	1.11 (*dd*, *J* = 4.0, 10.8), 2.27–2.23 (*m*)	27.5 (*t*)	2	2, 3, 5, 9, 10, 20
CH_2(2)	1.55–1.51 (*m*), 1.64–1.61 (*m*)	18.9 (*t*)	1, 3	1, 4, 10
CH_2(3)	1.06 (*dd*, *J* = 2.0, 10.8), 2.19 (*d*, *J* = 10.8)	33.7 (*t*)	2	1, 2, 5, 18, 19
C(4)		36.4 (*s*)		
H–C(5)	2.51 (*s*)	53.0 (*d*)	6	4, 6, 7, 9, 18–20
H–C(6)	5.04 (*d*, *J* = 4.3)	79.9 (*d*)	7	4, 5, 7,8, 10, 20
H–C(7)	3.98 (br. *s*)	71.8 (*d*)	6	5, 6, 8, 9, 14, 15
C(8)		45.3 (*s*)		
H–C(9)	1.89 (br. *dd*, *J* = 8.0)	48.8 (*d*)	11	1, 5, 8, 11, 12, 15, 20
C(10)		38.3 (*s*)		
CH_2(11)	1.61–1.59 (*m*), 1.72–1.70 (*m*)	28.9 (*t*)	9	8, 9, 12, 13, 16
H–C(12)	2.29–2.27 (*m*)	37.2 (*d*)	11	9, 13–17
CH_2(13)	1.55–1.57 (*m*), 1.61–1.59 (*m*)	27.5 (*t*)	12, 14	8, 11, 16
CH_2(14)	1.24 (*dd*, *J* = 7.2, 11.6), 2.39 (*ddd*, *J* = 6.4, 8.8, 11.6)	29.2 (*t*)	13	7, 9, 12, 15
CH_2(15)	2.27–2.23 (*m*), 2.93 (*d*, *J* = 16.2)	40.6 (*t*)		7, 9, 12, 14, 16, 17
C(16)		151.6 (*s*)		
CH_2(17)	4.95 (*d*, *J* = 1.4), 4.86 (*d*, *J* = 1.4)	107.3 (*t*)		12, 15, 16
Me(18)	1.32 (*s*)	26.1 (*q*)		3, 4, 5, 19
CH_2(19)	3.97 (*d*, *J* = 1.0), 3.72 (*d*, *J* = 11.0)	64.0 (*t*)		3, 4, 5, 18
C(20)		180.6 (*s*)		

[a]) At 500 MHz. [b]) At 125 MHz.

The ^{13}C-NMR (DEPT) spectrum (*Table*) exhibited signals corresponding to one Me, eight CH_2 (one being oxygenated), and five CH (two being oxygenated) groups, four quaternary C-atoms, including one COO group (δ(C) 180.6), and two olefinic

resonances (δ(C) 107.3 (*t*), 151.6 (*s*)). Considering the type of diterpenes previously isolated from this plant complex, in combination with analyses of the MS fragmentation pattern and characteristic NMR signals, the basic skeleton of **1** was identified as an *ent*-atisane diterpene [8][9].

By comparing the MS fragment ions and ^{1}H- and ^{13}C-NMR data of **1** with those of spiramilactone [9], the two compounds were found to have the same molecular weight and similar skeletons, but greatly differed in terms of the NMR chemical shifts for C(6), C(7), C(15), C(19), and C(20). The ^{1}H,^{1}H-COSY plot of **1** revealed the presence of three fragments (*A*–*C*; *Fig. 1,a*): $CH_2(1)-CH_2(2)-CH_2(3)$ (*A*), CH(5)–CH(6)O–CH(7)OH (*B*), and $CH(9)-CH_2(11)-CH(12)-CH_2(13)-CH_2(14)$ (*C*). The above evidence helped us to assigned C(6) and C(7) as two oxygenated CH moieties, as confirmed by the HMBC correlations of H–C(6) with C(4), C(5), C(7), C(8), and C(10), and of H–C(7) with C(5), C(6), C(8), C(9), C(14), and C(15) (*Fig. 1,a*).

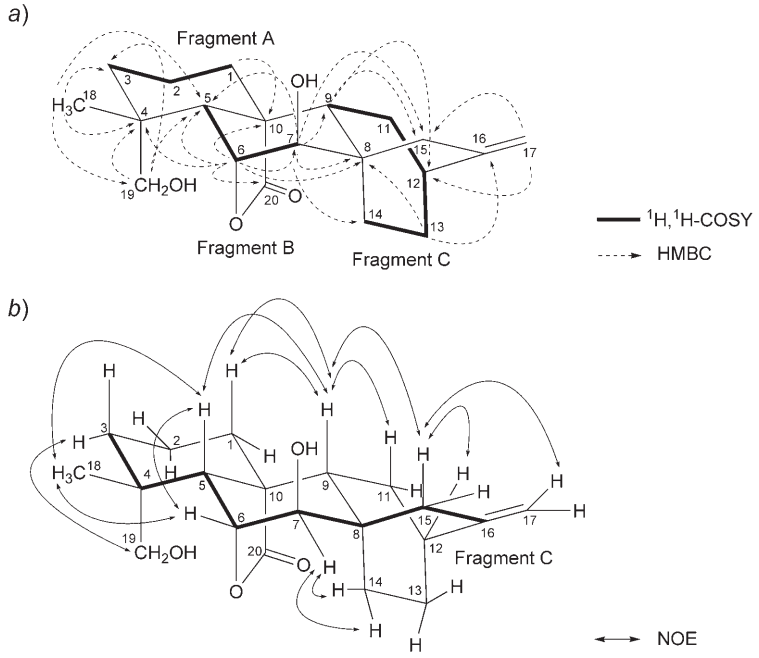


Fig. 1. a) *Key ^{1}H,^{1}H-COSY and HMBC correlations for* **1**; b) *key ROESY correlations for* **1**

γ-Lactone formation between C(6) and C(20) was supported by a correlation of H–C(6) to C(20) in the HMBC spectrum. There were HMBC cross-peaks between CH_2(17) (δ(H) 4.86, 4.95) and a CH_2 group (δ(C) 40.6), and between Me(18) (δ(H) 1.32) and an oxygenated CH_2 (δ(C) 64.0), which were, thus, assigned to C(15) and C(19), respectively.

The relative configuration of **1** was deduced from a ROESY experiment (*Fig. 1,b*). The NOE correlations of H–C(6) with both H–C(5) and Me(18), and of H–C(7) with CH_2(14) indicated β-configuration for both H–C(6) and the 7-OH group. The

structure and relative configuration of **1** were further supported by single-crystal X-ray crystallography (*Fig. 2*).

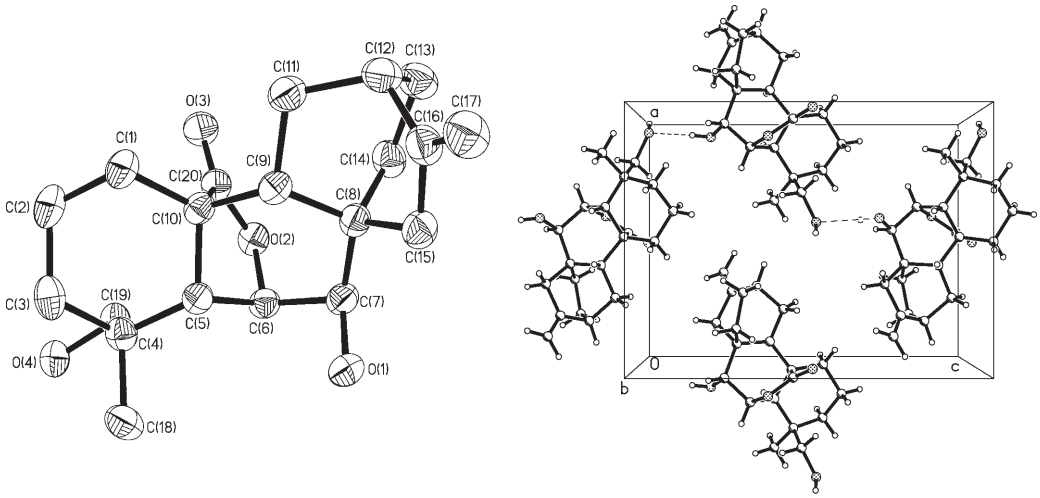


Fig. 2. *X-Ray crystal structure of spiramilactone E* (**1**)

From the above data, the structure of **1** was unequivocally determined as 7*β*,19-dihydroxy-*ent*-atis-16-en-6*α*,20-olide (= *rel*-(3*S*,4a*R*,4b*R*,8*R*,9*R*,10*R*,10a*R*)-decahydro-10-hydroxy-8-(hydroxymethyl)-8-methyl-2-methylidene-1*H*,5*H*-9,4b-(epoxymethano)-3,10a-ethanophenanthren-14-one).

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh, 10–40 μm; *Qingdao Marine Chemical Co.*) or *Sephadex LH-20* (*Pharmacia*). TLC: silica-gel plates; visualization by spraying with 10% H_2SO_4 in EtOH, followed by heating. Melting points (m.p.): *XRC-1* apparatus; uncorrected. Optical rotations: *Jasco DIP-370* digital polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AM-400* (400/100 MHz) and *Bruker DRX-500* (500/125 MHz) instruments; δ in ppm, *J* in Hz. EI-MS: *VG AutoSpec-3000* mass spectrometer; in *m/z*. HR-ESI-MS: *API Qstar-Pulsar LC/TOF* instrument.

Plant Material. The roots of *S. japonica* var. *acuta* YU were collected in Lijiang (Yunnan Province, P. R. China) in August 2000, and identified by Prof. *Hang Sun*. A voucher specimen (No. 8018) was deposited at the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The powdered, dried roots (6 kg) were extracted with 95% EtOH at reflux for 2 h (3 × 20 l). After removal of the solvent *in vacuo*, the crude extract was separated into basic and neutral parts, as described previously [10]. The neutral part (100 g) was purified by CC (SiO_2; $CHCl_3$/acetone 1:0 → 0:1) to afford five fractions (*Fr. A–E*). *Fr. B* (5 g) was separated by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to afford four subfractions (*Fr. B.1–B.4*). *Fr. B.3* was further purified by CC (SiO_2; petroleum ether/AcOEt 2:1) to yield **1** (9 mg).

Spiramilactone E (= *7β,19-Dihydroxy*-ent-*atis-16-en-6α,20-olide*; **1**). Colorless, lumpish crystals. M.p. 194–195° (AcOEt/$CHCl_3$ 4:1). $[\alpha]_D^{13} = -43.3$ ($c = 0.39$, $CHCl_3$/MeOH). IR (KBr): 3414, 2934, 1754, 1645, 1434, 1319, 1027, 948, 881. ^{1}H- and ^{13}C-NMR: see *Table*. EI-MS: 332 (2, M^+), 314 (2,

$[M - H_2O]^+$), 302 (100), 284 (30), 256 (40), 239 (48), 227 (19), 182 (22), 151 (28), 121 (30), 91 (38), 79 (36). HR-ESI-MS: 355.1888 ($[M + Na]^+$, $C_{20}H_{28}NaO_4^+$; calc. 355.1885).

X-Ray Crystal Structure of **1**[1]). Formula $C_{20}H_{28}O_4$; M_r 332.42 g/mol; crystal dimension: 0.30 × 0.40 × 0.40 mm; crystal system: orthorhombic; space group $P2_12_12_1$; unit-cell dimensions: $a = 10.834(1)$, $b = 11.184(1)$, $c = 15.532$ Å; $V = 1760.8(6)$ Å^3; $Z = 4$; $D_x = 1.254$ g/cm^3. Data were collected on a *MAC DIP-2030K* diffractometer, with a graphite monochromator (ω-2θ scans, $2\theta_{max} = 50.0°$), MoK_α radiation. The total number of independent and observed reflections was 1648 ($|F|^2 \geq 2\sigma|F|^2$). The structure was solved by direct methods, using SHELXS-97, expanded with difference *Fourier* techniques, and refined with NOMCSDP, using full-matrix least-squares calculations. Final indices: $R_1 = 0.0433$, $wR_2 = 0.1101$, $S = 1.091$, $(\Delta/\sigma)_{max} = 0.040$, $(\Delta\rho)_{min} = -0.152$ e/Å^3, $(\Delta\rho)_{max} = 0.181$ e/Å^3. H-Atoms were fixed at their calculated positions.

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[1]) The crystallographic data of **1** have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication number CCDC-640929. Copies of the data can be obtained, free of charge, at http://www.ccdc.cam.ac.uk/data_request/cif.