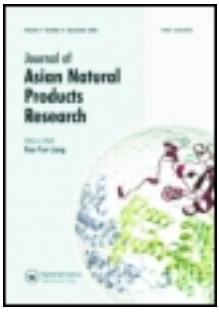

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# Structural Revision of Four Spiramine Diterpenoid Alkaloids from the Roots of Spiraea Japonica

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# STRUCTURAL REVISION OF FOUR SPIRAMINE DITERPENOID ALKALOIDS FROM THE ROOTS OF *SPIRAEA JAPONICA*

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On the basis of detailed $^{1}$H-NMR, $^{13}$C-NMR spectral analysis, especially by 2D NMR experiments ($^{1}$H–$^{1}$H COSY, HMQC, HMBC, and NOESY) as well as by chemical transformations, four isoatisine type diterpenoid alkaloids, spiramines P and Q, and U and T, have been reassigned as the 6*β* hydroxyl and 6*β* acetoxyl substituents, respectively, rather than the previously assigned 15*α* counterparts in our further studies on chemical constituents of the roots of *Spiraea japonica* var. *acuta*.

*Keywords:* *Spiraea japonica* var. *acuta*; Rosaceae; Diterpenoid alkaloids; Structural revision

## INTRODUCTION

*Spiraea japonica* var. *acuta* is one of the most common and abundant Rosaceae species in western regions of Yunnan province, China. In our previous papers [1–9], we reported twenty-two new structures of the spiramine series, spiramines A–V, isolated from *Spiraea japonica* var. *acuminata* Franch, *Spiraea japonica* var. *acuta* Yu, and *Spiraea japonica* var. *insisa* Yu. Among them, the structures of spiramines P, Q, T, and U were proposed as **1**, **2** [5], **3**, and **4** [7], respectively. In our previous paper concerning the structure elucidation of spiramines P (**1**) and Q (**2**), one of the hydroxyl groups

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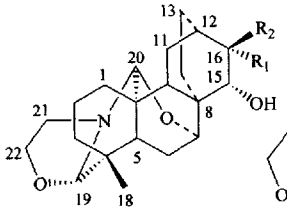
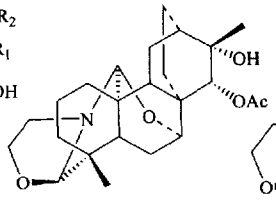
was erroneously assigned as 15$\alpha$ substituent by comparison of the $^{13}$C-NMR data with those of spiramine C (**5**) [9] since the hydroxyl-bearing methine signal was recorded as a broad singlet in the $^{1}$H-NMR spectrum [5]. Later in another paper [7], structures of spiramines T (**3**) and U (**4**) were determined by comparison of their $^{1}$H- and $^{13}$C-NMR data with those of spiramines P (**1**) and Q (**2**). In our continuing studies on biologically active and/or structurally novel compounds from *Spiraea japonica* and its varieties, we recently reexamined the chemical constituents of *Spiraea japonica* var. *acuta*, collected in another place of Li Jiang, Yunnan province. Apart from spiramines A (**6**) and B (**7**) [9], we also obtained spiradine F (**8**) [10] and spiramines P (**1**) and U (**4**). Detailed NMR analysis, including 2D NMR experiments and chemical transformations, resulted in the structural revision of spiramines P, Q, T, and U to structures **9**–**12**, rather than structures **1**–**4**, respectively. This paper describes the structural revision of these four diterpenoid alkaloids.

## RESULTS AND DISCUSSION

The 95% ethanol extract of the roots of *Spiraea japonica* var. *acuta* was treated in the usual procedures [9] to give alkaloid fraction which was subjected to repeated column chromatography on silica gel (petroleum ether–acetone–diethylamine, gradient) to afford spiramines A and B [9], spiradine F [10,11], spiramine P [5] and spiramine U [7].




**1** $R_1 = CH_3$, $R_2 = OH$
**2** $R_1 = OH$, $R_2 = CH_3$
**5** $R_1$, $R_2 = CH_2$

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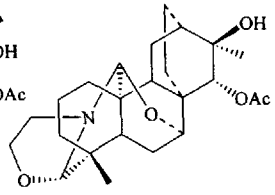


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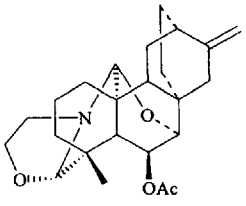
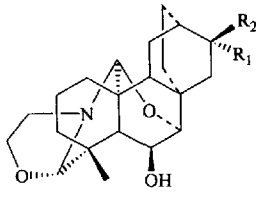
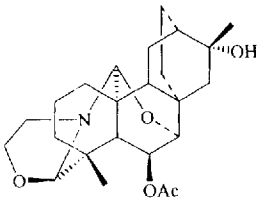



**8**




**9** $R_1 = CH_3$, $R_2 = OH$
**10** $R_1 = OH$, $R_2 = CH_3$

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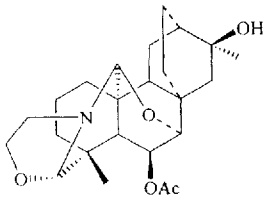


12

The molecular formula of spiramine U, obtained as colorless crystals, was determined as $C_{24}H_{35}O_5N$ (417.2500, calcd. for $C_{24}H_{35}O_5N$, 417.2515) by high resolution EIMS. The IR spectrum showed absorption bands for hydroxyl (3531 cm $^{-1}$), ester carbonyl (1723 cm $^{-1}$), together with ether linkage (1028, 1040 cm$^{-1}$). The isoatisine-type skeleton for spiramine U was established by comparison of its $^1$H- and $^{13}$C-NMR data with those reported [9,12,13]. The $^1$H- and $^{13}$C-NMR data were assigned on the basis of $^1$H–$^1$H COSY, DEPT, HMQC, and HMBC experiments (Tables I and II). The $^1$H-NMR spectrum clearly exhibited the presence of two tertiary methyl groups at $\delta$ 1.07 (3H, s, H-18) and 1.36 (3H, s, H-17), one acetyl methyl group at $\delta$ 2.00 (3H, s, –OAc), and an oxazolidine ring system at $\delta$ 3.63, 3.43, 3.26, and 3.17 (each 1H, m, H-22a, 22b, 21a, and 21b) and $\delta$ 3.83 (1H, s, H-19), among them the latter suggested the S-configuration of C-19 in the oxazolidine ring of spiramine U [9,14]. Meanwhile, two hydroxyl-bearing methine proton signals at $\delta$ 3.53 (1H, d, $J$ = 4.9 Hz) and $\delta$ 5.61 (1H, dd, $J$ = 2.0, 4.9 Hz) were assignable to H-7$\beta$ and H-6$\alpha$, respectively, and these designations were further confirmed by $^1$H–$^1$H COSY spectrum which showed clearly cross peak of the two protons. Similarly H-5 ($\delta$ 1.08, d, $J$ = 2.0 Hz) also showed cross peak with H-6 in $^1$H–$^1$H COSY spectrum. The above data confirmed that the hydroxyl bearing methine proton must be linked to C-6 in spiramine U. The other identified cross peaks in $^1$H–$^1$H COSY spectrum were listed in Table I.

One bond correlations between $^1$H and $^{13}$C nuclei were established by HMQC experiment, which provided valuable evidence to establish the structure of spiramine U. The signals of C-6, C-7, C-19 and C-20 in the $^{13}$C-NMR spectrum at $\delta$ 70.8 (d), 70.8 (d), 94.6 (d), and 85.4 (d) correlated with the corresponding protons in the $^1$H-NMR spectrum at $\delta$ 5.61, 3.53, 3.83 and 4.54. The oxazolidine ring carbons C-21 and C-22 resonated in the $^{13}$C-NMR spectrum at $\delta$ 51.0 (t) and 63.3 (t) correlated with the protons at $\delta$ 3.26, 3.17, and $\delta$ 3.63, 3.43, respectively. Three methyl carbons resonated at $\delta$ 31.3 (C-17), 22.5 (C-18) and 21.2 (–OAc) were coupled with protons at $\delta$ 1.36, 1.07 and 2.00, respectively, in the HMQC spectrum.

TABLE I NMR assignments and $^1H-^1H$ COSY, HMQC, and HMBC correlations of **12***

| *Atom no.* | *$^1H$-NMR* (400 MHz) | | *$^{13}C$-NMR* (100 MHz) | | | |
|---|---|---|---|---|---|---|
| | *δ (J in* Hz) | *$^1H-^1H$ COSY* | *δ* | *DEPT* | *HMQC* | *HMBC (H → C)* |
| 1 | 1.68 (1H, m)<br>1.18 (1H, m) | 2 | 29.2 | $CH_2$ | H-1 | 2, 3, 9, 10, 20 |
| 2 | 2.03 (1H, m)<br>1.40 (1H, m) | 1, 3 | 20.2 | $CH_2$ | H-2 | 1, 3 |
| 3 | 1.46 (1H, m)<br>1.20 (1H, m) | 2 | 40.6 | $CH_2$ | H-3 | 1, 2, 4, 5 |
| 4 | | | 35.4 | C | | |
| 5 | 1.08 (1H, d,<br>$J = 2.0$) | 6, 19, 20 | 52.8 | CH | H-5 | 3, 4, 6, 7, 10, 19, 20 |
| 6 | 5.61 (1H, dd,<br>$J = 2.0, 4.9$) | 5, 7 | 70.8 | CH | H-6 | 4, 5, 7, 8, 10, 23 |
| 7 | 3.53 (1H, d,<br>$J = 4.9$) | 6 | 70.8 | CH | H-7 | 5, 6, 8, 9, 15, 20 |
| 8 | | | 36.4 | C | | |
| 9 | 1.53 (1H, m) | 11 | 43.2 | CH | H-9 | 5, 8, 10, 11, 14, 15, 20 |
| 10 | | | 35.2 | C | | |
| 11 | 1.50 (1H, m)<br>1.31 (1H, m) | 9, 12 | 22.7 | $CH_2$ | H-11 | 8, 9, 10, 12, 13, 16 |
| 12 | 1.51 (1H, m) | 11, 13 | 39.0 | CH | H-12 | 9, 11, 13, 14, 15, 16, 17 |
| 13 | 1.91 (1H, m)<br>1.38 (1H, m) | 12, 14 | 21.2 | $CH_2$ | H-13 | 11, 12, 14, 16 |
| 14 | 1.80 (1H, m)<br>1.21 (1H, m) | 13 | 26.3 | $CH_2$ | H-14 | 8, 13, 15 |
| 15[a] | 1.90 (1H, dd,<br>$J = 3.4, 12.4$)<br>1.28 (1H, d,<br>$J = 12.4$) | 14 | 47.4 | $CH_2$ | H-15 | 8, 9, 14, 16, 17 |
| 16 | | | 72.4 | C | | |
| 17 | 1.36 (3H, s) | 15 | 31.3 | $CH_3$ | H-17 | 12, 15, 16 |
| 18 | 1.07 (3H, s) | 5 | 22.5 | $CH_3$ | H-18 | 3, 4, 5 |
| 19 | 3.83 (1H, s) | 5 | 94.6 | CH | H-19 | 3, 5, 20 |
| 20 | 4.54 (1H, s) | 5 | 85.4 | CH | H-20 | 5, 7, 9, 10, 19, 21 |
| 21 | 3.26 (1H, m)<br>3.17 (1H, m) | 22 | 51.0 | $CH_2$ | H-21 | 19, 20, 22 |
| 22 | 3.63 (1H, m)<br>3.43 (1H, m) | 21 | 63.3 | $CH_2$ | H-22 | 19, 21 |
| 23 | | | 169.4 | C | | |
| 24 | 2.00 (3H, s) | | 21.2 | $CH_3$ | | 23 |

*Using $CDCl_3$ as solvent, δ in ppm.
[a]The H-15β proton showed W-type coupling ($J = 3.4$ Hz) with one of the H-14 protons.

Long-range $^1H-^{13}C$ chemical shift correlation (HMBC) provided further conclusive structural evidence for spiramine U. Some selected HMBC correlations are shown in Fig. 1. The observed cross peaks between H-6 and C-4, C-5, C-7, C-8, C-10 and C-23, between H-7 and C-5, C-6, C-8, C-9, C-15, and C-20, and between H-17 and C-12, C-15, and C-16 supported structure **12** for spiramine U.

TABLE II NMR assignments and $^1H$–$^1H$ COSY, HMQC, and HMBC correlations of **9***

| *Atom no.* | *$^1H$-NMR* (400 MHz) | | *$^{13}C$-NMR* (100 MHz) | | | |
|---|---|---|---|---|---|---|
| | *δ (J in Hz)* | *$^1H$-$^1H$ COSY* | *δ* | *DEPT* | *HMQC* | *HMBC (H → C)* |
| 1 | 1.32 (1H, m)<br>1.21 (1H, m) | 2 | 29.6 | $CH_2$ | H-1 | 2, 3, 9, 10, 20 |
| 2 | 2.26 (1H, m)<br>1.39 (1H, m) | 1, 3 | 20.9 | $CH_2$ | H-2 | 1, 3 |
| 3 | 1.40 (1H, m)<br>1.52 (1H, m) | 2 | 41.3 | $CH_2$ | H-3 | 1, 2, 4, 5 |
| 4 | | | 35.8 | C | | |
| 5 | 1.38 (1H, br, s) | 6, 19, 20 | 56.8 | CH | H-5 | 3, 4, 6, 7, 19, 20 |
| 6 | 5.09 (1H, dd,<br>*J* = 2.1, 4.9) | 5, 7 | 69.1 | CH | H-6 | 4, 5, 7, 8, 10 |
| 7 | 3.70 (1H, d,<br>*J* = 4.9) | 6 | 75.2 | CH | H-7 | 5, 6, 8, 9, 14, 15, 20 |
| 8 | | | 37.5 | C | | |
| 9 | 2.03 (1H, dd,<br>*J* = 2.9, 10.5) | 11 | 43.5 | CH | H-9 | 5, 8, 11, 14, 15, 20 |
| 10 | | | 36.0 | C | | |
| 11 | 1.60 (1H, m)<br>1.23 (1H, m) | 9, 12 | 23.3 | $CH_2$ | H-11 | 9, 12, 16 |
| 12 | 1.83 (1H, m) | 11, 13 | 40.0 | CH | H-12 | 9, 11, 13, 14, 15, 16, 17 |
| 13 | 2.65 (1H, m)<br>1.48 (1H, m) | 12, 14 | 22.3 | $CH_2$ | H-13 | 11, 12, 14, 16 |
| 14 | 2.12 (1H, m)<br>1.50 (1H, m) | 13 | 27.8 | $CH_2$ | H-14 | 8, 15 |
| 15[a] | 3.06 (1H, dd,<br>*J* = 3.2, 12.4)<br>1.89 (1H, d,<br>*J* = 12.4) | 14 | 48.9 | $CH_2$ | H-15 | 8, 9, 14, 16, 17 |
| 16 | | | 71.7 | C | | |
| 17 | 1.71 (3H, s) | 15 | 32.0 | $CH_3$ | H-17 | 12, 15, 16 |
| 18 | 1.40 (3H, s) | 5 | 23.3 | $CH_3$ | H-18 | 3, 4, 5, 19 |
| 19 | 3.91 (1H, s) | 5 | 95.4 | CH | H-19 | 3, 5, 20, 22 |
| 20 | 4.64 (1H, s) | 5 | 85.5 | CH | H-20 | 5, 7, 9, 10, 19, 21 |
| 21 | 3.38 (1H, m)<br>3.18 (1H, m) | 22 | 51.5 | $CH_2$ | H-21 | 20 |
| 22 | 3.75 (1H, m)<br>3.40 (1H, m) | 21 | 63.4 | $CH_2$ | H-22 | 19, 21 |

*Using $C_5D_5N$ as solvent, δ in ppm.
[a]The H-15β proton showed W-type coupling (*J* = 3.2 Hz) with one of the H-14 protons.

The relative stereochemistry of **12** was deduced by NOE interactions from a NOESY experiment. H-5 signal showed cross peaks both to H-18 and H-7, suggesting H-5, H-7, and H-18 in the syn- and β-configuration. Furthermore, the observed cross peaks between the C-6 acetoxyl methyl signal to H-5 and H-7 proton signals also indicated the β-configuration for C-6 acetoxyl group.

To provide chemical evidence for the structure of spiramine U, chemical transformations were also conducted (see Fig. 2). The known compound

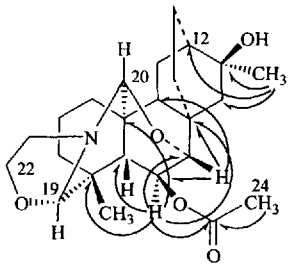

FIGURE 1 Selected HMBC correlations of spiramine U.

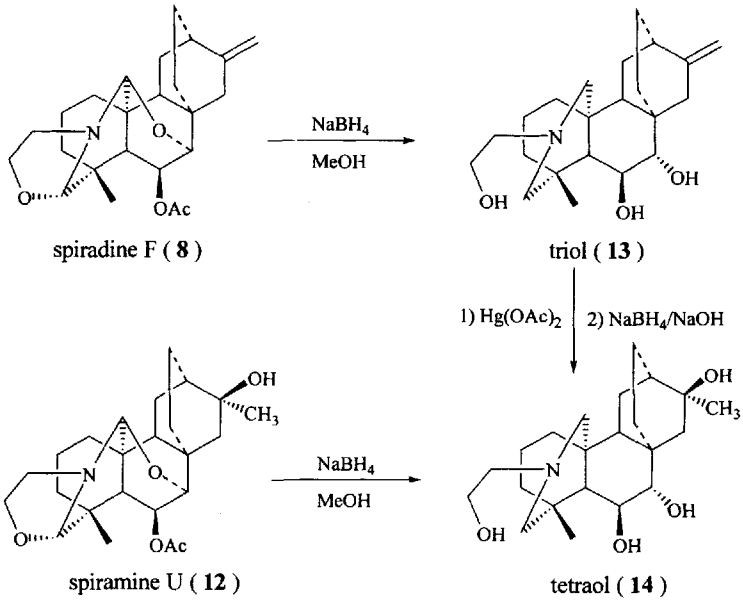


FIGURE 2 Chemical transformations of spiradine F and spiramine U.

spiradine F (**8**) was reduced with sodium borohydride [10] to give a triol (**13**), the exo-carbon–carbon double bond of the triol (**13**) was then hydrated by the oxymercuration–demercuration procedure [15] to give a tetraol (**14**). Similarly, mild reduction of spiramine U with sodium borohydride also gave the tetraol (**14**), suggesting that spiradine F and spiramine U possess the same substitution pattern in ring B.

From all of above mentioned spectral and chemical evidence, the structure of spiramine U should be concluded as (**12**), not as (**4**) in our previous

report [7]. It should be pointed out that the structure of spiramine U (**12**) has been previously reported as a C-19 epimeric mixture (1 : 1) from *Thalictrum sessile*, named as thalicsiline [16,17], while it should be regarded as a pure compound in our present study. It should also be mentioned that the $^{13}$C-NMR data of the methene signals, C 1–3, 11, 13–15 for thalicsiline, reported by Wu *et al.*, should be reassigned according to our present 2D NMR study.

After the structural confirmation of spiramine U, it is easier for us to revise the structure of spiramine P. The elemental formula of spiramine P was determined by high resolution EIMS as $C_{22}H_{33}O_4N$ (375.2386, calcd. for $C_{22}H_{33}O_4N$, 375.2409). The complete $^1$H- and $^{13}$C-NMR connectivity was also established by extensive use and interpretation of $^1$H–$^1$H COSY, HMQC, and HMBC NMR spectra (Table II), which provided unequivocally structural revision of spiramine P from **1** to **9**.

Some structural and spectral features of spiramine P are worthly of mention. The existence of two coupled one-proton signals, which appeared as a doublet at $\delta$ 3.70 ($J = 4.9$ Hz) and a doublet of doublets at $\delta$ 5.09 ($J = 2.1$, 4.9 Hz) in the $^1$H-NMR spectrum, were clearly observed in $^1$H–$^1$H COSY spectrum. These two protons are assignable to H-7$\beta$ and H-6$\alpha$, respectively. Moreover, the signals of C-4, C-5, C-7, C-8, C-10 in the $^{13}$C-NMR spectrum at $\delta$ 35.8 (s), 56.8 (d), 75.2 (d), 37.5 (s), 36.0 (s) correlated with the same proton signal at $\delta$ 5.09 (H-6$\alpha$) in HMBC spectrum, confirmed that one of the hydroxyl groups was attached to C-6 position. Some other significant cross peaks in the HMBC spectrum include H-7 and C-5, C-6, C-8, C-9, C-14, C-15, C-20, and between H-17 and C-12, C-15, C-16 (see Fig. 3). Thus, the structure of spiramine P should be represented as **9**, rather than **1**.

Although we have not isolated spiramines Q (**2**) [5] and T (**3**) [7] in our present study, we suggest here that their structures should also be revised in the same way to structures **10** and **11**, respectively, by detailed analysis of the reported NMR data.

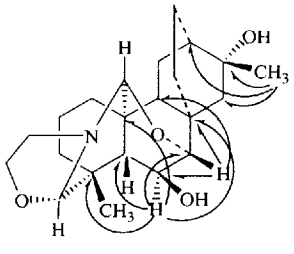


FIGURE 3 Selected HMBC correlations of spiramine P.

## EXPERIMENTAL

### General Experimental Procedures

Melting points were determined using a Kofler micro-melting point apparatus and are not corrected. Optical rotations were obtained on a HORIBA SEPA-300 High Sensitive Polarimeter. IR spectra were recorded as KBr tablets with a Bio-Rad FTS-135 spectrometer. EIMS were measured on a VG Auto Spec-3000 spectrometer with direct inlet as 70 eV. 1D and 2D NMR were taken on a Bruker AM-400 and DRX-500 spectrometer using TMS as internal standard. TLC was performed on TLC plates precoated with Silica gel $F_{254}$ (Qingdao Haiyang Chemical Ltd.). Solvents were distilled prior to use.

### Plant Material

The roots of *Spiraea japonica* var. *acuta* were collected in Li Jiang, northwestern Yunnan province, in July, 1998, and the specimen was identified by Prof. Zheng-Wei Lu of Kunming Botanical Garden. The specimens (9807L) were deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

### Extraction and Isolation

The air-dried roots of *Spiraea japonica* var. *acuta* (200 kg) were extracted with 95% ethanol in reflux condition for two times in a semi-plant scale equipment in Kunming Institute of Botany. The extracts were condensed *in vacuo* to give a crude residue (12 kg) which was dissolved in 3% HCl solution and filtered. The acidic solution was basified with 5% aqueous NaOH to pH 11 and then extracted with $CHCl_3$. Evaporation of $CHCl_3$ solution gave 1.2 kg of crude alkaloid fraction which was subjected to column chromatography on silica gel. Elution was carried out with mixtures of solvents of increasing polarity starting with petroleum ether–acetone–diethylamine. The fractions eluted with petroleum ether–acetone–diethylamine (40 : 10 : 1) were combined and concentrated and then further purified by repeated flash column chromatography using petroleum ether–ethyl acetate–methanol (32 : 4 : 1) to afford the major compounds **6** (3.5 g), **7** (3.0 g), **8** (20 g), and a minor constituent (50 mg), whose structure is still under determination. Compound **9** (300 mg) and **12** (1.0 g) were obtained from the fractions eluted with petroleum–acetone–diethylamine (20 : 10 : 1).

*Spiramine U* (**12**) This was obtained as colorless crystals, m.p. 215–217°C; $[\alpha]_D^{27}$ −112.5 (c 0.35, $CHCl_3$); IR (KBr) $\gamma_{max}$ 3531, 2925, 2880, 1723, 1460, 1372, 1242, 1204, 1121, 1094, 1040, 1028, 1007, 935, 894 $cm^{-1}$; $^1H$-, $^{13}C$-NMR, and 2D NMR data, see Table I; EIMS: *m/z* 417 $[M]^+$ (100), 400 (7), 389 (24), 374 (62), 72 (38); HREIMS *m/z* 417.2500 (calcd. for $C_{24}H_{35}O_5N$, 417.2515).

*Spiramine P* (**9**) Colorless crystals, m.p. 239–240°C; $[\alpha]_D^{27}$ −44.3 (c 0.30, $CHCl_3$); IR (KBr) $\gamma_{max}$ 3443, 2936, 2909, 2882, 1463, 1406, 1371, 1205, 1119, 1098, 1037, 1023, 981, 909, 873 $cm^{-1}$; $^1H$-, $^{13}C$-NMR, and 2D NMR data, see Table II; EIMS *m/z* 375 $[M]^+$ (90), 346 (50), 319 (75), 278 (35), 180 (100), 92 (50), 72 (78); HREIMS *m/z* 375.2386 (calcd. for $C_{22}H_{33}O_4N$, 375.2409).

*Mild reduction of spiradine F* (**8**) Sodium borohydride (600 mg) was added to a solution of 500 mg of spiradine F (**8**) in 50 ml of $CH_3OH$ and the reaction mixture was stirred at room temperature for 3 h. Removal of the solvents under reduced pressure gave a residue which was partitioned between $H_2O$ and $CHCl_3$. The organic fraction was taken to dryness and chromatographed over a silica gel column (mobile phase, $CHCl_3$ : $CH_3OH$ 12 : 1) to give a triol (**13**) (389 mg, yield 86%), whose structure was identified by spectral analysis. Triol (**13**): IR (KBr) $\gamma_{max}$ 3367, 2943, 2856, 1656, 1456, 1434, 1373, 1101, 1083, 1040 $cm^{-1}$; $^1H$-NMR (400 MHz, $CDCl_3$): $\delta$ 4.75 (1H, d, $J$ = 2.2 Hz, H-17a), 4.60 (1H, d, $J$ = 2.2 Hz, H-17b), 3.89 (1H, t, $J$ = 9.9 Hz, H-6$\alpha$), 3.65 (2H, t, $J$ = 6.2 Hz, H-22), 3.09 (1H, d, $J$ = 9.2 Hz, H-7$\beta$), 2.45–2.63 (4H, m, H-19, 20), 1.07 (3H, s, H-18); $^{13}C$-NMR (100 MHz, $CDCl_3$): $\delta$ (DEPT) 40.0 ($CH_2$, C-1), 23.0 ($CH_2$, C-2), 42.3 ($CH_2$, C-3), 34.8 (C, C-4), 53.1 (CH, C-5), 69.6 (CH, C-6), 82.7 (CH, C-7), 38.2 (C, C-8), 36.4 (CH, C-9), 38.2 (C, C-10), 28.2 ($CH_2$, C-11), 48.4 (CH, C-12), 26.5 ($CH_2$, C-13), 22.8 ($CH_2$, C-14), 45.3 ($CH_2$, C-15), 150.5 (C, C-16), 106.0 ($CH_2$, C-17), 29.2 ($CH_3$, C-18), 60.7 ($CH_2$, C-19), 54.2 ($CH_2$, C-20), 57.8 ($CH_2$, C-21), 60.8 ($CH_2$, C-22); EIMS *m/z* 361 $[M]^+$ (8), 344 (5), 331 (45), 330 (100), 314 (80), 286 (65), 241 (32), 199 (30), 132 (42).

*The oxymercuration–demercuration of triol* (**13**) One hundred mg of mercuric acetate was dissolved in 15 ml of $H_2O$. To this solution, 100 mg of triol (**13**) in 10 ml of THF was added. The reaction mixture was refluxed for 1 h and then NaOH solution (60 mg NaOH in 5 ml $H_2O$) and $NaBH_4$ solution (25 mg $NaBH_4$ in 5 ml MeOH solution) were added to the reaction mixture. The reaction mixture was filtered. After removal of the solvents (THF) *in vacuo*, $CHCl_3$ was added. The organic layer was washed with water, dried with $Na_2SO_4$ and filtered, then taken to dryness under reduced pressure and chromatographed over a silica gel column (mobile phase, $CHCl_3$ : $CH_3OH$

7 : 1) to give a tetraol (**14**) (48.3 mg, yield 46%), whose structure was identified by spectral analysis. Tetraol (**14**): $^1$H-NMR (400 MHz, $CDCl_3$): $\delta$ 4.31 (1H, t, $J=9.9$ Hz, H-6$\alpha$), 4.05 (2H, t, $J=6.2$ Hz, H-22), 3.40 (1H, d, $J=9.5$ Hz, H-7$\beta$), 3.06 (2H, m, H-21), 2.57–2.79 (4H, m, H-19, 20), 1.54 (3H, s, H-17), 1.43 (3H, s, H-18); $^{13}$C-NMR (100 MHz, $CDCl_3$): $\delta$ (DEPT) 41.1 ($CH_2$, C-1), 22.7 ($CH_2$, C-2), 43.9 ($CH_2$, C-3), 35.4 (C, C-4), 54.1 (CH, C-5), 69.3 (CH, C-6), 83.2 (CH, C-7), 39.4 (C, C-8), 39.0 (CH, C-9), 40.4 (C, C-10), 25.6 ($CH_2$, C-11), 48.1 (CH, C-12), 23.2 ($CH_2$, C-13), 22.6 ($CH_2$, C-14), 55.3 ($CH_2$, C-15), 71.7 (C, C-16), 31.4 ($CH_3$, C-17), 30.2 ($CH_3$, C-18), 62.4 ($CH_2$, C-19), 55.5 ($CH_2$, C-20), 60.6 ($CH_2$, C-21), 62.5 ($CH_2$, C-22); EIMS *m/z* 379 $[M]^+$ (22), 378 $[M-1]^+$ (30), 361 (75), 349 (96), 348 (90), 331 (86), 330 (100), 316 (25), 131 (15).

*Mild reduction of spiramine U* (**12**) Spiramine U (**12**) was also reduced with sodium borohydride in the same procedures as those for spiradine F to give a tetraol whose $^1$H-NMR, EIMS data and co-TLC behavior are the same as the authentic sample tetraol (**14**).

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