CHEMISTRY OF DENUDATINE V.[1] REARRANGEMENT REACTIONS OF DENUDATINE

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Abstract -- Treatment of denudatine **1** with 10% HCl solution at 30-50 °C leads to rearrangement to two pairs of epimers **(5A)** and **(5B)**, **(8A)** and **(8B)** as well as **7** and **15**. The rearrangement mechanisms are discussed. Conversion of **7→5A/5B→15** under acid catalysis can be realized. While the conversion of **15** to **5A/5B** can be realized with NaOH-DMF at 120-126 °C. Structures for **5A/5B**, **7**, **8A/8B** and **15** were established on the basis of chemical and spectral methods.

INTRODUTION

In 1961, Singh first isolated denudatine from the roots of *Delphinium denudatum* Wall.[2] Pharmacological studies on denudatine showed that it possesses a marked inhibition of the isolated rabbit duodenal strip and stimulation on guinea pig uterus strips as well as prophylactic inhibition of cardiac arrhythmia caused by aconitine in rats.[3,4] Structure of denudatine has been proved as **1** based on chemical and spectral methods as well as single crystal X-ray diffraction analysis.[3,5-8] In 1980, Wiesner *et al.* reported the total synthesis of denudatine derivatives diacetyloxodenudatine and dihydrodenudatine.[9] Except for some chemical degradations about denudatine,[5] chemical studies on denudatine has not been reported yet. About 20 g of denudatine **(1)** as the major alkaloid isolated from the roots (8.5 kg) of *Aconitum nagarum* Stapf *var. lasiandrum* W. T. Wang abled us to study its chemistry. In the course of this research, acidic rearrangement products of denudatine **(1)** and the interesting reactions of **1** with NBS-HOAc have been found and reported as communications.[1,10-12] In this paper, we report details on these rearrangement products of **1** and their possible reaction mechanisms, the interconversions among these compounds as well as a study on the ^{1}H- and ^{13}C-NMR spectra for compounds **(5A)**, **(5A/5B)**, **(8A/8B)**, **(7)** and **(15)** based on their 2D-NMR spectra .

RESULTS AND DISCUSSION

Treatment of C_{20}-diterpenoid alkaloids having the allylic secondary alcohol systems, e.g., atisine[13] and garryfoline,[14] with acids usually leads to rearrangement to the 16β-methyl ketone derivatives. Attempt to

Dedicated to Prof. Xiao Tian Liang on the occasion of his 74 th birthday.

bring about this transformation of **1** under similar conditions (30-50 °C, 10% HCl solution, 1-3 d) failed. The products of this reaction are more complicated, surprisingly, a pair of epimers **(5A)** and **(5B)** (72% yield), and **7** (10% yield) and **15** (10% yield) being formed.

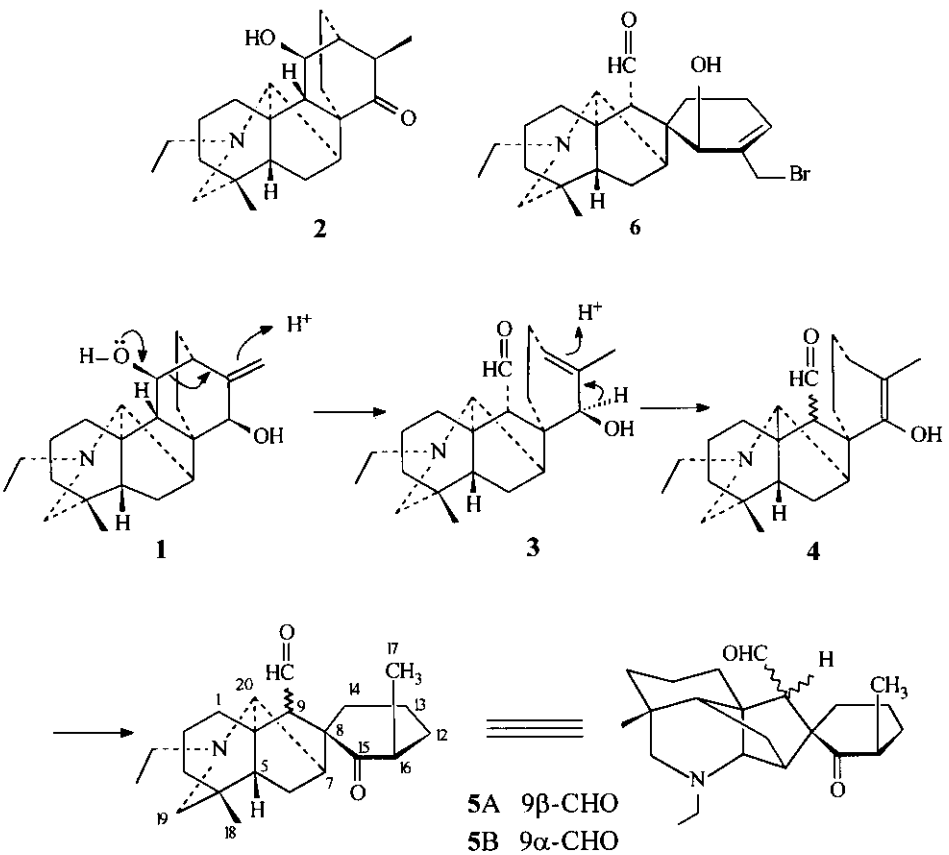

Figure 1

Compounds **(5A/5B),** $C_{22}H_{33}NO_2$ (HREIMS: *m/z* 343.2521, required 343.2482), are a pair of epimers obtained in an amorphous form. The IR spectrum of **5A/5B** exhibited the presence of carbonyl groups (1705 and 1703 cm^{-1}). While their mass spectra showed the important ion fragments at *m/z*(%): 328 (M–CH_3, 5), 315 (M-CO, 75), 314(M-CHO, 75), 286(100). ^1H NMR(200 MHz) spectrum of **5A/5B** displayed the following signals: δ 0.75(0.70)(3H, s, 4–CH_3), 0.98(1.00)(3H, d, J=6.4 Hz, 16–CH_3), 1.10(hidden, NCH_2CH_3) and 9.98(9.65) (1H, d, J=5 Hz, CHO). The ^{13}C-NMR spectrum of the epimers **(5A/5B)** revealed the presence of three methyl, nine methylene, six methine and four quaternary carbons. Structures of the epimeric compounds have been deduced as **5A** and **5B** based on their spectral data. In order to unambiguously differentiate their chemical shifts, separation of the mixture of **5A** and **5B** using column chromatography on silica gel afforded pure compound **(5A)**, then ^1H-NMR and ^{13}C-NMR spectra of this compound were measured. Because of displaying the NOE relationships between the 7-H (δ

2.80/~1.40 ppm) and the methyl group (δ 0.98/1.00 ppm) at C-16 in the NOEDS experiments, configuration of the methyl group at C-16 in both **5A** and **5B** has been deduced as the β-direction. The 9β-configuration of the CHO group in **5A** has been proposed based on the fact that there is significant difference (4.3 ppm) of values of the chemical shift of the aldehyde carbonyl carbon(C-11) between **5A** (δ 202.0 ppm) and an analogue **(6)**[12](δ 206.3 ppm) and the NOE experiments. When the signal at δ 9.91 ppm(11-CHO) was irradiated, the enhancements of the signals at δ ~1.71 ppm(1β-H) and ~2.00 ppm(5β-H) were observed. Monitoring of the reaction products using a silica gel TLC revealed that in the initial phase the major product of the rearrngement is **5B** having the 9α-CHO group. It was converted partially to **5A** with 9β-CHO group depending upon the reaction process until the final equilibration between both **5A** and **5B**. At this time, the proportion of **5A** and **5B** is about 2.5:1 based on the intergration of the CHO signals in the ^{1}H-NMR spectrum. Assignments of all the signals of ^{13}C- and ^{1}H-NMR spectra for **5A** and **5B** (Tables 1, 2) have been carried out mainly on the basis of 2D-NMR(^{1}H COSY, HETCOR, HMBC, COLOC), 1D NOEDS and comparison with denudatine **(1)**[15] and diacetyldenudatine **(12)**. As shown in Figure 1, the possible mechanism of this unusual rearrangement involves initial formation of **3**, followed by an allylic rearrangement *via* protonation of the double bond to form **4**, which is isomerized to **5A** and **5B**. In this rearrangement from **1** to **5A** and **5B**, the epimerization at C-9 has happened.

Compound **(7)**, $C_{22}H_{33}NO_2$ (HREIMS: *m/z* 343.2522, required 343.2482), was obtained in a white amorphous form. The infrared spectrum of **7** showed the characteristic peaks at 3405 cm^{-1} for the hydroxyl group. In its mass spectrum, there are important ion fragments at 328(M-Me, 100) and *m/z* 326(M-OH). The ^{1}H-NMR spectrum of **7** exhibited the presence of one *N*-ethyl, two methyls(δ 0.74, s, 4-Me; 1.80, d, J=11 Hz, 16-Me) and one olefinic proton(δ 5.59, d, J=6 Hz, 12-H). The one-proton singlet at δ 5.22 ppm and broad singlet at δ 4.18 ppm were attributed to the protons at C-11 and C-15, respectively. The ^{13}C-NMR spectrum combined with DEPT spectra of **7** showed the presence of 22 signals for the 22 carbons present in the molecule, of which there are three methyl, eight methylene, seven methine and four quaternary carbons. A doublet signal at δ 99.1 ppm comes from a hemiacetal group was assigned to C-11. Therefore, structure of this secondary rearrangement product may be deduced as **7** based on the aforementioned evidence. Because the formation of a hemiacetal-containing ring C in **7** requires 9β-CHO and 15β-OH groups,[12] the α-direction of 9-H in **7** could be deduced. This was also supported by the fact that the chemical shifts for C-9 in ^{13}C-NMR spectra of both **7** (δ 61.3 ppm) and **9** (δ 61.8 ppm) are very close to each other. In addition, the α-direction of the hydroxyl group at C-11 in **7** thus may be deduced only on the basis of comparison with nmr data for **9**.[11] Assignments of all the signals of NMR spectra for **7** have been carried out on the basis of 2D-NMR spectral studies for **8A/8B** (Table 3). It is interesting to note that when treatment of **1** with 10% HCl solution containing a little ethyl alcohol at 30-40 °C leads to only a pair of epimers **(8A)** and **(8B)** (48% yield) besides the *starting material*.

Compounds **(8A/8B)** are a mixture of a pair of epimers obtained in an amorphous form with the common molecular formula $C_{24}H_{37}NO_2$ (HREIMS: *m/z* 371.2845, required 371.2795). The mass spectra for **8A/8B** showed the important ion fragments at *m/z* 356(M-Me) and *m/z* 342 (M-CH_2CH_3). Their ^{1}H-NMR (200 MHz) spectra exhibited the presence of the following signals: δ 0.74(3H, s, 4-Me), 1.05 (3H, t, J=7

Hz, NCH_2CH_3), 1.74(3H, s, 16-Me) and 5.52 (1H, d, J=5 Hz, 12-H). ^{1}H- and ^{13}C-NMR spectra of the epimers also displayed the characteristic signal for an *O*-ethyl group [at δ 1.07(3H, ABX_3 system, t, J=7 Hz, OCH_2CH_3), 3.34(1H, ABX_3 system, dq, J_1=9 Hz, J_2=7 Hz, $OHCHCH_3$), 3.58(1H, ABX_3 system, dq, J_1=9 Hz, J_2=7 Hz, $OHCH\text{-}CH_3$); δ 14.7(15.3)(OCH_2CH_3), 61.4(OCH_2CH_3)]. From these data, structure of the epimers may be deduced as **8A** and **8B**. All the ^{13}C- and ^{1}H-NMR data obtained for **8A/8B** (Table 3) support structure **(8A/8B)**. A possible mechanism for the unusual rearrangements from **1** to **7** or **8A/8B** is shown in Figure 2. The reaction process involves initially breakage of C(11)-C(12) bond, followed by conversion of **10** to **11** *via* inversion of the 9α-formyl group at C-9, finally, condensation of the 9β-CHO and 15β-OH group in **11** to form **7** or **8A/8B**.

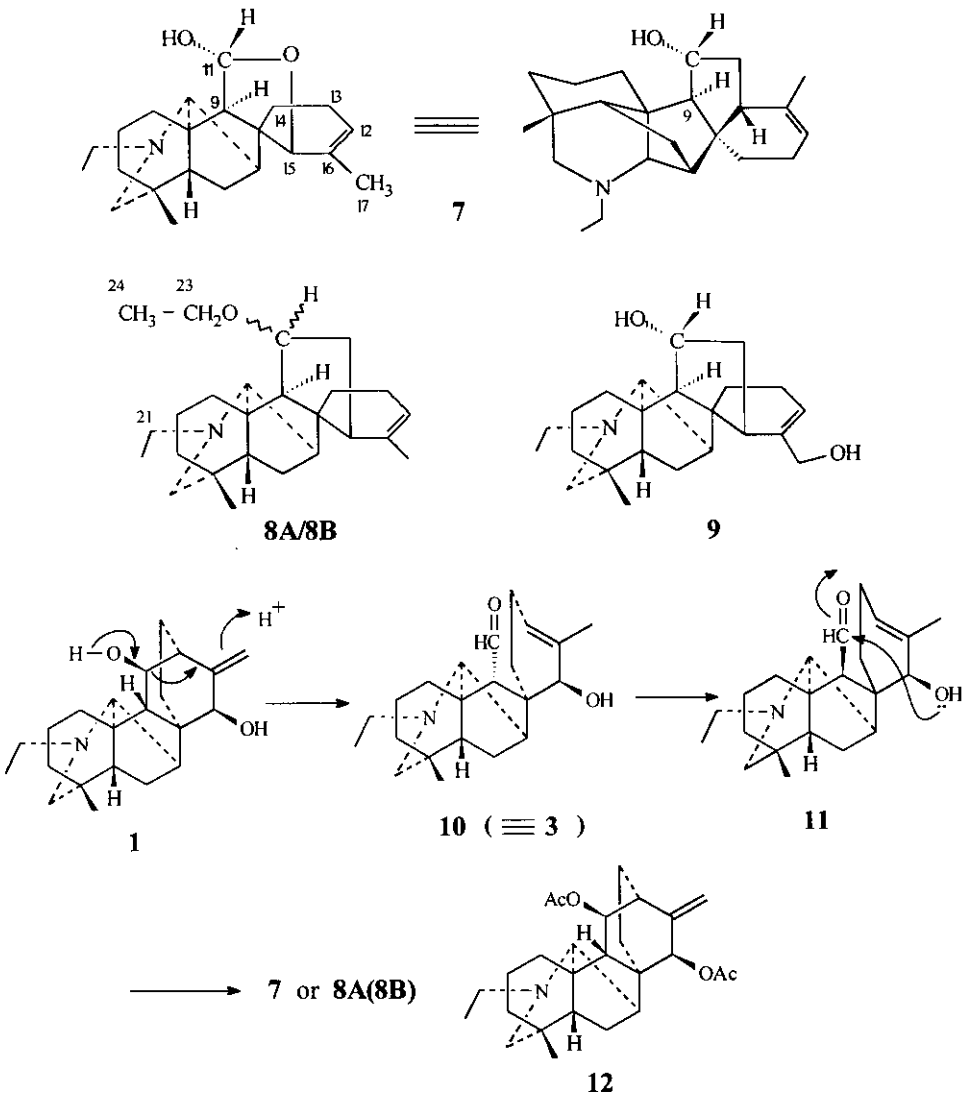

Figure 2

Table 1 NMR data for **5A** (400 MHz for ^1H, 100 MHz for ^{13}C, $CDCl_3$)

Carbon	^{13}C	^1H	^1HCOSY	HMBC(H→C)	COLOC(H→C)
1	41.7t	~1.71m	~2.04, ~1.40		
		~2.04m	~1.71		
2	20.2t	~1.40m	~1.70, ~1.50		
		~2.38m			
3	24.6t	~0.77m	~1.50		
		~1.50m	~0.77, ~1.40		
4	34.1s	---	---		
5	43.5d	2.00m	~1.18		
6	39.4t	~1.18m	~1.55, 2.00		
		~1.55m	~1.18	C_4, C_5, C_{10}, C_{20}	
7	40.4d	2.80d(5.4)	~1.55		
8	66.2s	---	---		
9	64.5d	~1.72d(hidden)	9.66		
10	48.6s	---	---		
11	202.1d	9.91d(5.2)	~1.72		C_9
12	20.4t	~1.62m	~1.28		
13	22.4t	~1.70m	~2.05		
14	37.1t	~1.28m	~1.62, ~2.05		
		~2.05m	~1.28		
15	215.0s	---	---		
16	43.5d	~2.55m	0.98		
17	14.6q	0.98d(6)	~2.55		
18	26.1q	0.70s	---		
		~2.20	~2.20		C_{20}
19	57.2t	ABq(hidden)			
		~2.60	~2.60		
20	69.9d	2.98 br s	---		
NCH_2CH_3	50.9t	~2.55m	1.09		
NCH_2CH_3	13.1q	1.09t(7)	~2.55		

Compound **(15),** $C_{22}H_{33}NO_2$ (HREIMS: *m/z* 343.2522, required 343.2482), was obtained in an amorphous form. The infrared spectrum of **15** showed the charactristic peak at 3390 cm^{-1} for the hydroxyl groups. In mass spectrum of **15**, the important ion fragments at *m/z* 315(M-CO, 98) and *m/z* 231(100) were observed. The ^1H-NMR(200 MHz, $CDCl_3$) spectrum of **15** exhibited the presence of one *N*-ethyl group at

δ 1.03 ppm(3H, t, J=7 Hz), two methyl groups at δ 0.73 ppm (s, 4-Me) and δ 0.95 ppm(s, 16-Me). The ^{13}C-NMR (50 MHz, $CDCl_3$) spectrum together with DEPT spectra of **15** displayed the presence of 22 signals for the 22 carbons in the molecule, of which three were methyl, nine methylene, five methine and five quaternary carbons including ketone signal at δ 225.0 ppm attributed to C-15. The structure of this rearrangement product was deduced as **15** based on the aforementioned spectral data. The α-configuration of the hydroxyl group at C-11 in **15** was proved by the presence of the nOe relationship between the 11β-H and the 16β-methyl group. While configuration of the 9α-H in **15** is also dictated by the constraint of cis B/C ring fusion since the ring B forms with C_{20} a rigid bicyclo [2.2.1]heptane system. By the way, treatment of **12** with 10% HCl solution under refluxing for 2-3 h. still leads to a little of **15**. All the ^{13}C- and ^{1}H-NMR as well as ^{1}H COSY data obtained for **15** (Tables 4,5) support structure **15.** A possible mechanism for formation of **15** from **1** or **12** was shown in Figure 3. It is interesting to note that this rearrangement shown in Figure 3 produced a new type of diterpenoid alkaloid having a five-membered ring C. In order to further understand these rearrangement of denudatine **(1)** mentioned above, we also studied the interconversion among the compounds **(5A/5B)**, **(7)** and **(15)**. when treatment of **7** with 10% HCl solution at 82-84 °C for 28 h, compounds **(5A/5B)** and a little of **15** were produced. Refluxing a mixture of **5A/5B** with 10% HCl solution for 3 days leads to almost quantitative conversion to **15**. Attempt to convert **15** to **5A/5B** with 10% HCl solution under vigorous conditions failed. But, treatment of **15** with NaOH-DMF at 122-126 °C overnight leads to **5A/5B** (ratio=1:1, totally 50% yield for both compounds) besides the starting material. Figure 4 showed the possible process of the aforementioned conversions. As shown in Figure 4, elimination of the hydroxyl group at C-11 in **15** by dehydration is blocked by the lack of active hydrogen flanking the ketonic carbonyl group.

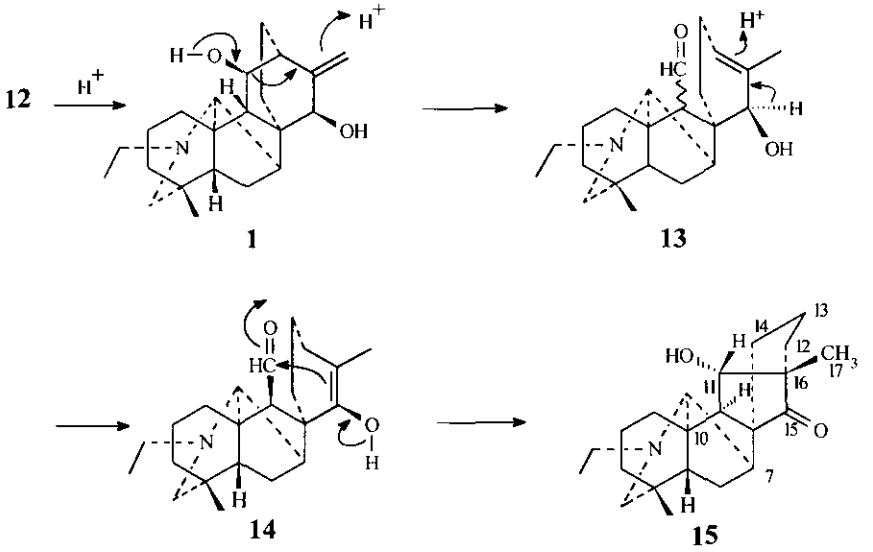

Figure 3

Table 2 NMR data for **5B** (400 MHz for ^1H, 100 MHz for ^{13}C, $CDCl_3$)

Carbon	^{13}C	^1H	^1HCOSY	HMBC(H→C)
1	41.2t	~2.72m	~1.72	
2	21.8t	~1.70m	~2.72	
3	25.1t	~1.10m	~1.55, ~1.7	
		~1.55m	~1.10, ~2.72	
4	34.4s	---	---	
5	49.3d	~1.25m	~1.28, ~1.55	C_{20}
6	37.0t	~1.28m	~1.25, ~1.55	
		~1.55m	~1.28, ~1.25	C_4
7	39.5d	~1.40m	~1.55	
8	60.9s	---	---	
9	57.2d	3.20d(6)	9.91	
10	47.3s	---	---	
11	204.6d	9.66d(4.8)	3.20	
12	27.0t	~1.35m	~2.0, ~2.0	
		~2.00m	~1.40, ~2.55	
13	20.0t	~1.40m		
		~2.38m		
14	35.0t	~1.25m	~1.40, ~2.2	
		~2.20m	~1.25	
15	213.2s	---	---	
16	43.2d	~2.55m	1.00, ~2.0	
17	15.0q	1.00d(6.4)	~2.55	
18	26.2q	0.75s	---	
		~1.95	~2.60	
19	56.8t	ABq(hidden)		
		~2.60	~1.95	
20	69.1d	3.50 br.s	---	
NCH_2CH_3	51.2t	~2.55m	1.09	
NCH_2CH_3	13.2q	1.12t(7)	~2.55	

Conversion from **15** to **5A/5B** under alkaline conditions may involve breakage between C-11 and C-16 bond *via* a retroaldol reaction to afford **16**, followed by isomerization to give **5A/5B**. The pssible rearrangement mechanism shown in Figures 1, 2 and 3 indicated that breakage between the C(11)-C(12)bond under acid conditions must be involved as shown in Figure 5. This is in agreement with those

in the rearrangements of hetisine and its derivatives[16,17] as well as other reactions reported in the literature.[12] This is the key reaction step to produce rearrangement products **(5A/5B)**, **(7)**, **(8A/8B)**, and **(15)**.

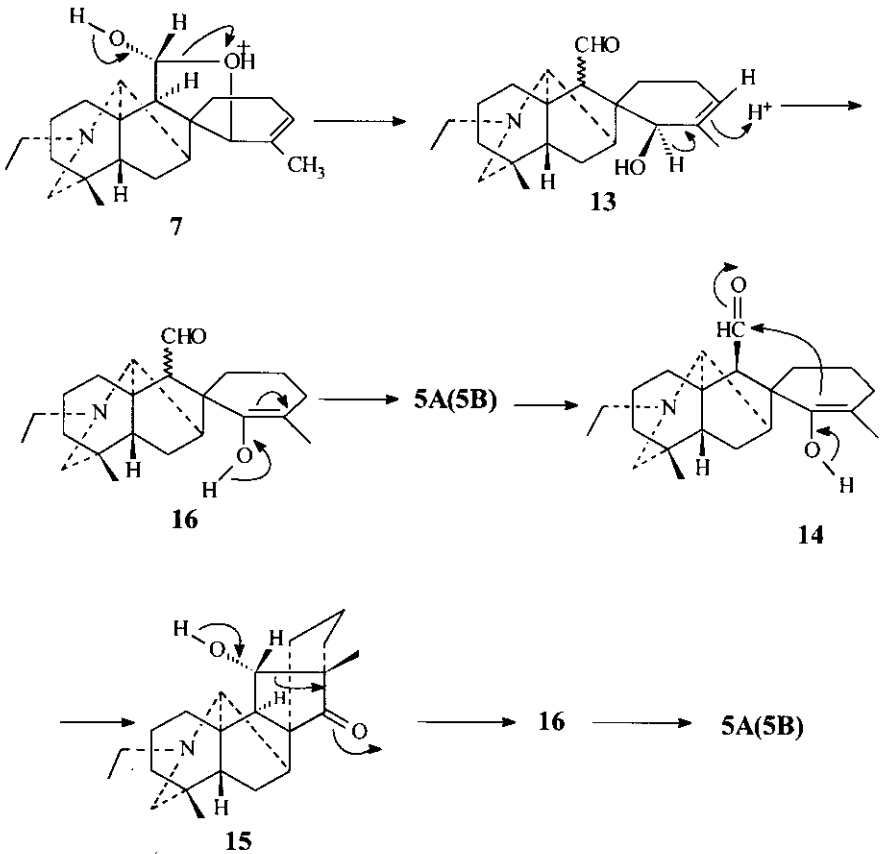

Figure 4

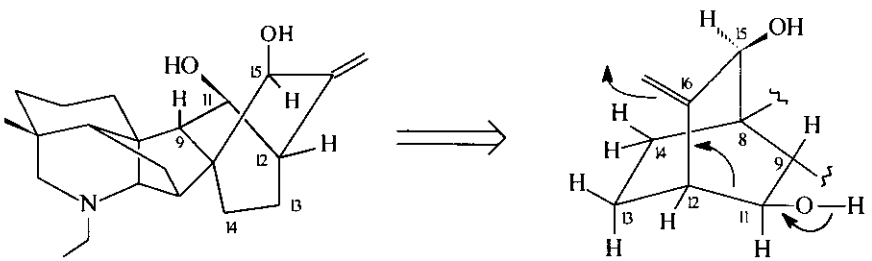

Figure 5

Table 3 NMR data for **8A/8B** (400 MHz for ^{1}H, 100 MHz for ^{13}C, $CDCl_3$)

Carbon	^{13}C	^{1}H	COSY	COLOC(H→C)
1	39.9t	~1.55m	~1.42	C_{20}
2	20.2t	~1.42m	~1.55, ~1.38, ~1.78	C_4, C_{10}
3	23.2t	~1.38m	~1.42, ~1.78	C_4
		~1.78m	~1.42, ~1.38	C_1, C_2
4	34.3s	---	---	
5	43.5d	~1.50m	~1.20	C_4, C_6, C_7,C_{18}
6	31.1t	~1.20m	~1.50	C_4
7	48.0d	~1.95m	~1.20	C_6, C_8
8	47.5s	---	---	
9	60.6(60.5)d	~1.90s	---	C_1, C_7, C_{10}, C_{14}, C_{23}
10	49.1s	---	---	
11	103.1(105.3)d	4.85(4.76)s	---	C_8, C_{10}, C_{15}, C_{23}
12	125.1(124.9)d	5.48d(5)	~1.74	C_{13}, C_{17}
13	32.7t	~1.42m	~1.74	C_8
		~1.74m	~1.42, 5.48	C_{15}, C_{16}
14	20.7t	~1.78m	~1.42, ~2.13	
		~2.13m	~1.78	C_{12}, C_{13}
15	81.6(81.7)d	4.14 br s	---	C_{11}, C_{12}, C_{15}, C_{16}, C_{17}
16	134.9(135.0)s	---	---	
17	21.0q	~1.75s	5.84	C_{16}
18	26.3q	0.71s	---	C_4, C_{19}
		2.23	2.38	C_4, C_{18}
19	57.6t	ABq(11.4)		
		2.38	2.23	
20	71.9d	3.02(3.18) br s	---	C_8, C_{19}
NCH_2CH_3	51.1t	2.40m	1.10	
NCH_2CH_3	13.1q	1.10t(7)	~2.40	
		3.30	1.04, 3.53	
OCH_2CH_3	61.4t	ABX_3/dq(9, 7)		
		3.53	1.04, 3.30	
OCH_2CH_3	14.6q	1.04ABX_3/t(7)	3.30, 3.53	C_{23}

Table 4 NMR data for **15** (300 MHz for ^{1}H, 75 MHz for ^{13}C, $CDCl_3$-CD_3OD)

Carbon	^{13}C	^{1}H	^{1}HCOSY
1	38.7t	~1.45m	~1.50, ~2.13, ~2.25
		~2.13m	~1.45, ~1.50, ~2.25
2	19.7t	~1.50m	~1.30, ~1.45, ~2.13, ~2.25
		~2.25m	~1.30, ~1.45, ~1.50, ~2.13
3	25.1t	~1.30m	~1.50, ~2.25
4	34.8s	---	---
5	41.4d	~1.30m	~1.16
6	29.3t	~1.16m	~1.15, ~1.30
7	39.0d	~1.15m	~1.16
8	57.5s	---	---
9	57.1d	~2.15d(hidden)	3.61
10	47.8s	---	---
11	73.0d	3.61d(6.3)	~2.15
12	41.4t	~1.30m	~1.85
13	39.5t	~1.85m	~1.40, ~1.95
14	17.9t	~1.40m	~1.85, ~1.95
		~1.95m	
15	225.0s	---	---
16	59.6s	---	---
17	17.1q	0.82s	---
18	26.1q	0.67s	---
19	57.6t	2.21 ABq(hidden) 2.75	2.75 2.21
20	72.0d	2.93 br s	---
NCH_2CH_3	50.8t	~2.45m	1.06, ~2.60
		~2.60	1.06, ~2.45
NCH_2CH_3	12.6q	1.06t(6.6)	~2.45, ~2.60

Table 5 ^{13}C NMR data for **15** (75 MHz, $CDCl_3$)

Carbon	$CDCl_3$	Carbon	$CDCl_3$
1	38.6	12	41.1
2	20.0	13	39.5
3	25.0	14	17.9

Table 5 (Continued)

Carbon	$CDCl_3$	Carbon	$CDCl_3$
4	34.5	15	225.0
5	42.2	16	57.5
6	30.2	17	17.1
7	39.1	18	26.1
8	57.5	19	57.3
9	57.3	20	72.0
10	47.9	NCH_2CH_3	50.8
11	73.9	NCH_2CH_3	12.6

EXPERIMENTAL

In general Melting points were determined on the Kofler block (uncorrected). IR spectra were measured on Nicol FT-IR 20 SXB in KBr pellets. Mass spectra were measured on HP 5988A (for LRMS) and Kratos ms 80 (for HRms) mass spectrometers. NMR spectra were determined on Bruker, AC-200, 300 MHz and JEOL 400 MHz NMR spectrometers in $CDCl_3$ with TMS as internal reference. Chromatographic silica gel G and silica gel H were purchased from Qingdao Haiyang Chemical Factory and silica gel G for TLC were treated with sodium hydroxide (absorbent-NaOH, 100:0.5). Centrifugal TLC were run with LBC-1 model instrument made in China on silica gel G plates. TLC were carried out on silica gel G with solvent systems S_1(chloroform-methanol, 9:1), S_2(ether-acetone, 95:5). A polyvinylsulfonic ion exchange resin (H-form, cross linking 1 × 3) from Chemical Works of Nankai University was used for the extraction of the total alkaloids.

Isolation of denudatine One hundred grams of the total alkaloids were obtained from the roots (8.5 kg) of *Aconitum nagarum* Stapf *var. lasiandrum* W. T. Wang according to the literature method.[18] The total alkaloids (100.0 g) were subdivided into four portions. Each one (25 g) was dissolved in 500 mL of 10% ammonium hydroxide to afford a large amount (8.5 g) of precipitate (crude denudatine), which was filtered and washed with a little methanol, then crystallized from methanol to obtain colorless needle crystals, mp 253-254 °C (5.1 g). Its IR spectrum showed the presence of the exocyclic double bond(1660 cm^{-1}) and the hydroxyl groups (3376, 3296 cm^{-1}). It was identified as denudatine **(1)** on the basis of comparison of co-TLC behavior and mmp with the authentic sample. The aforementioned operations were repeated to give 20 g of denudatine.

Preparation of compounds **(5A/5B), (7), (8A/8B)** and **(15)**

1). A mixture of one gram of denudatine **1** and 60 mL of 10% HCl was heated on water bath for 30 min, cooled and alkalized with concd. ammonium hydroxide, then extracted with chloroform (200 mL × 2). This was separated using column chromatogeraphy over silica gel H (50 g), eluting with chloroform-methanol (99:1, 98:2, 8:2) to give **5A** (160 mg), a mixture of **5A/5B** (320 mg) as well as **15** (70 mg).

2). To the 200 mg of denudatine **(1)** 13 mL of 10% HCl solution was added, then the solution was stirred at 40-50 °C for 3 d, alkalization of the reaction solution with concd. ammonium hydroxide, followed by extraction with chloroform (20 mL × 2), then concentration gave a residue, which was

separated on column chromatography over silica gel H (15 g), eluting with chloroform-methanol (95:5) to give **7** (8 mg) besides a mixture of **5A/5B** (65 mg).

3). A solution of denudatine **(1)** (200 mg) and 30 mL of 10% HCl solution was allowed to stand at rt for several days. After this, a little of ethyl alcohol was added to the reaction solution and heated on water bath (*ca.* 50 °C) for 1 h. The reaction solution was alkalized to pH 8 with concd. ammonium hydroxide and extracted with chloroform(50 mL × 2). Concentration of the combined chloro-form extraction solution gave a residue(170 mg), which was separated on a preparative TLC over silica gel G, developing with chloroform-methanol (99:2) to afford a mixture of **8A/8B** (95 mg).

Preparation of **12** A solution of denudatine **(1)** (200 mg, 0.58 mmol), acetic anhydride(3 mL, 32 mmol) and anhydrous pyridine (3 mL) was allowed to stand at rt overnight, then treatment of the reaction solution in a general operation gave a residue, which was passed through a small column packed with silica gel H, eluting with chloroform-methanol (99:1) to give a pure colorless prisms compound **(12)** (190 mg, 77.0%). **12**: mp 134~135 °C, IR cm^{-1}: 1742, 1721(OAc). ^{1}H-NMR(200 MHz): δ 0.71(3H, s, 4-Me), 1.05(3H, t, J=7 Hz, NCH_2CH_3), 2.05, 2.17(each, 3H, s, 2 × OAc), 3.44(1H, br s, 20-H), 4.84(1H, d, J=10 Hz, 11α-H), 4.94(2H, br s, 17-H_2), 5.41(1H, t, J=2 Hz, 15α-H). ^{13}C-NMR (50 MHz): δ 39.7(1), 20.2(2), 27.3(3), 33.9(4), 50.2 (5), 25.6(6), 43.2(7), 42.9(8), 51.7(9), 45.2(10), 73.9(11), 41.9(12), 23.6(13), 22.2(14), 77.7(15), 147.2(16), 110.2(17), 26.4(18), 56.9(19), 70.9(20), 51.0(21), 12.9(22), 170.5, 170.8, 21.3, 21.4(2 × OAc). EI-MS (*m/z*, %): 427($M^{+\bullet}$, 15), 368(M-OAc, 100). **12** was identified as diacetyldenudatine based on comparison of co-TLC behavior and IR spectra with the authentic sample.[19]

Compound **(5A)** was obtained as an amorphous powder, ^{1}H-NMR(200 MHz): δ 0.72(3H, s, 4-Me), 0.96(3H, d, J=6.4 Hz, 16-Me), 9.91(1H, d, J=5 Hz, 9β-CHO). ^{13}C-NMR(50 MHz): δ 41.6t(1), 20.2t(2), 24.4t(3), 33.9s(4), 43.2d*(5), 39.4t(6), 41.0d(7), 66.2s(8), 64.4d(9), 48.5s(10), 202.2d(11), 29.4t(12), 22.3t(13), 36.8t(14), 214.9s(15), 43.5d*(16), 14.4q(17), 26.0q(18), 57.1t(19), 69.9d(20), 50.5t(NCH_2-), 13.1q(NCH_2CH_3). NOEDS (400 MHz) (η): 1-H(δ 9.91 ppm) to 1β-H (δ ~1.71 ppm, 3.0%) and 5β-H(δ 2.00 ppm, 7.0%). Anal. Calcd for $C_{22}H_{33}NO_2$: C 76.96, H 9.62, N 4.07. Found: C 76.52, H 9.01, N 3.82.

Compounds **(5A/5B)** was obtained as an amorphous powder, IR cm^{-1}: 1705(1703)(CHO). HR-MS: calcd for $C_{22}H_{33}NO_2$ 343.2482, found 343.2521. EI-MS (*m/z*, %): 343($M^{+\bullet}$, 18), 328(M-15, 5), 315(M-CO, 75), 314(M-CHO, 75), 286(100). ^{1}H-NMR (200 MHz): δ 0.75(0.70)(3H, s, 4-Me), 0.98(1.00)(3H, d, J=6.4 Hz, 16-Me), *ca.*1.10(NCH_2CH_3, hidden), 9.93(9.65)(1H, d, J=5 Hz, CHO). NOEDS (η): 16-CH_3(δ 0.98/1.00 ppm) to 7-H [δ 2.80(8%)/~1.40 ppm(6.2%)]; 7-H (δ 2.80/~1.40 ppm) to 16-CH_3 [δ 0.98(8%)/ 1.00 ppm(12%)]. ^{13}C-NMR** (50 MHz): δ 41.6(39.4)t(1), 20.0(21.8)t(2), 24.4(25.0)t(3), 33.9(34.3)s(4), 43.5*(49.5)d(5), 39.4(36.9)t(6), 41.0(40.3)d(7), 66.2(66.1)s(8), 64.4(57.3)d(9), 48.5(47.3)s(10), 202.2 (204.5)d(11), 29.4(26.9)t(12), 22.3(20.1)t(13), 36.8(35.0)t(14), 214.9(213.1)s(15), 43.2*(43.4)d(16), 14.6(15.0)q(17), 26.0(26.1)q(18), 57.1(56.8)t(19), 69.9(69.1)d(20), 50.5(51.2)t(NCH_2-), 13.1(13.3)q (NCH_2CH_3). ^{1}H(400 MHz)- and ^{13}C(100 MHz)-NMR data for **5A** and **5B** see in Tables 1 and 2, respectively. Anal. Calcd for $C_{22}H_{33}NO_2$: C 76.96, H 9.62, N 4.07. Found: C 76.40, H 9.20, N 3.91.

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* Assignments may be exchanged.

** Assignments for some ^{13}C-signals in the literature 10 are incorrect and here were revised.

Compound **(7)** was obtained as an amorphous powder, IR cm^{-1}: 3408(OH). HR-MS: calcd for $C_{22}H_{33}NO_2$ 343.2482, found 343.2522. EI-MS(*m/z*, %): 343($M^{+\cdot}$, 69), 342(M-1, 29), 328(M-Me, 100), 326(M-OH, 11), 296(12), 254(10). ^{1}H-NMR(200 MHz): δ 0.74(3H, s, 4-Me), 1.07(3H, t, J=7 Hz, NCH_2CH_3), 1.80(3H, d, J=11 Hz, 16-Me), 3.03(1H, br s, 20-H), 4.18(1H, br s, 15α-H), 5.22(1H, s, 11β-H), 5.59(1H, d, J=6 Hz, 12-H). ^{13}C-NMR(50 MHz): δ 40.1t(1), 20.2t(2), 23.2t(3), 34.3s(4), 43.5d(5), 31.2t(6), 47.8d(7), 48.0s(8), 61.3d(9), 49.4s(10), 99.7(100.5)d(11), 124.8t(12), 32.1t(13), 20.8t(14), 81.7d(15), 138.2s(16), 20.9q(17), 26.3q(18), 57.7t(19), 71.9d(20), 50.9t(NCH_2-), 13.2q(NCH_2CH_3). Anal. Calcd for $C_{22}H_{33}NO_2$: C 76.96, H 9.62, N 4.07. Found: C 76.32, H 9.15, N 3.84.

Compound **(8A/8B)** was obtained as an amorphous powder, IR cm^{-1}: 3040(OH). HR-MS: calcd for $C_{24}H_{37}NO_2$ 371.2795, found 371.2845. EI-MS(*m/z*, %): 371($M^{+\cdot}$), 356(M-Me), 342(M-CH_2CH_3), 219. ^{1}H-NMR(200 MHz): δ 0.74(3H, s, 4-Me), 1.05(3H, t, J=7 Hz, NCH_2CH_3), 1.07(3H, ABX_3 system, t, J=7 Hz, OCH_2CH_3), 1.74(3H, br s, 16-Me), 3.02(3.22)(1H, ABX$_3$, dq, J_1=9 Hz, J_2=7 Hz, O-HCHCH$_3$), 4.18(1H, br s, 15α-H), 4.89(4.80)(1H, s, 11-H), 5.52 (1H, d, J=5 Hz, 12-H). ^{13}C-NMR(50MHz): δ 40.1t (1), 20.3t(2), 23.2t(3), 34.3s(4), 43.6(43.1)d(5), 31.2(29.6)t(6), 48.0(48.1)d(7), 47.5s(8), 60.7(60.6)d(9), 49.4s(10), 103.2(105.3)d(11), 125.1(125.0)d(12), 32.6(32.8)t(13), 20.8t(14), 81.6(81.7)d(15), 135.0 (135.2)s(16), 21.1q(17), 26.3q(18), 57.7t(19), 72.1(72.0)(20), 50.8t(NCH_2-), 13.4(14.0)q(NCH_2CH_3), 61.4t(OCH_2CH3), 14.7(15.3)q(OCH_2CH_3). ^{1}H(300 MHz)- and ^{13}C(75 MHz)-NMR data for **8A/8B** see in Table 3. Proportion of **8A** and **8B** is about 4:1 based on the integration of the 11-H signals in the ^{1}H-NMR spectrum. Anal. Calcd for $C_{24}H_{37}NO_2$: C 77.62, H 9.96, N 3.77. Found C 77.02, H 9.31, N 3.12.

Compound **(15)** was obtained as an amorphous powder, IR cm^{-1}: 3390(OH), 1742(C=O). HR-MS: calcd for $C_{22}H_{33}NO_2$ 343.2482, found 343.2522. EI-MS(*m/z*, %): 343($M^{+\cdot}$, 44), 315(98), 231(100). ^{1}H-NMR(200 MHz, $CDCl_3$): δ 0.73(3H, s, 4-Me), 0.95 (3H, s, 16-Me), 1.03 (3H, t, J=7 Hz, NCH_2CH_3), 2.08(1H, br s, 20-H), 3.79(1H, d, J=7 Hz, 11β-H). NOEDS(η): 11-H(δ 3.39ppm) to 16-CH_3(δ 0.95 ppm)(8.4%); 16-CH_3 to 11-H (19.0%). ^{1}H(300 MHz, $CDCl_3$): δ 0.72(3H, s, 4-Me), 0.95(3H, s, 16-Me), 1.06(3H, t, J=6.6 Hz, NCH_2CH_3), 2.87(1H, br s, 20-H), 3.76(1H, d, J=6.6 Hz, 11β-H). ^{13}C-NMR(50 MHz, $CDCl_3$): δ 38.9t(1), 20.3t(2), 25.9t(3), 34.5s(4), 42.8d(5), 30.8t (6), 39.2d(7), 57.8s(8), 57.7d(9), 47.9s(10), 74.3d(11), 41.5*t(12), 41.0*t (13), 18.2t(14), 225.0s(15), 60.8s(16), 17.3q(17), 26.5q(18), 57.6t(19), 72.0d(20), 50.6t(NCH_2-), 13.2q(NCH_2CH_3). ^{1}H(300 MHz)- and ^{13}C(75 MHz)-NMR data for **15** see in Tables 4 and 5, respectively. Anal. Calcd for $C_{22}H_{33}NO_2$: C 76.96, H 9.62, N 4.07. Found: C 76.21, H 9.21, N 3.75.

Conversion of 7 to **5A/5B** Treatment of 3 mg of **7** with 1 mL of 10% HCl solution at 82-84 °C for 28 h gave a mixture (3 mg), which showed the major products of **5A/5B** on silica gel G TLC plates, developing with both solvent systems(S_1 and S_2).

Conversion of **5A/5B** to **15** A solution of 10 mg of **5A/5B** in 5 mL of 10% HCl solution was refluxed for 3 d. Treatment of the reaction solution in general operation gave a residue (8 mg), which exhibited a spot as the major product corresponding to **15** on silica gel G TLC plates, developing with both solvent systems(S_1 and S_2).

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* Assignments may be exchanged.

Conversion of **15** to **5A/5B** Treatment of 10 mg of **15** with 10 mL of DMF and 0.1 g of sodium hydroxide at 122-126 °C for 24 h showed spots corresponding to mixture (9 mg) of **5A** and **5B** besides the starting material on silica gel G TLC plates, developing with both solvent systems(S_1 and S_2).

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