Tortifoline, a Novel (20*S*,22*R*)-5α-Cevanine Alkaloid from *Fritillaria tortifolia*

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A new D/E *cis* (20*S*,22*R*,25*S*)-20-deoxy-5α-cevanine alkaloid, tortifoline (1), was isolated from the Chinese herbal drug, "takuri baimo" (dried bulbs of *Fritillaria tortifolia*). The structure of 1 was deduced from spectral data and its absolute configuration was confirmed by X-ray crystallographic analysis.

Keywords tortifoline; *Fritillaria tortifolia*; Liliaceae; delavine; chuanbeinone; (20*S*,22*R*,25*S*)-20-deoxy-5α-cevanine

"Bei-mu", an important Chinese medicine, is the dried bulbs of *Fritillaria* genus (Liliaceae). Many *Fritillaria* plants are cultivated in China and utilized as an antitussive, expectorant, or sedative, and for other purposes.[1] We have been studying the alkaloids of many kinds of "Bei-mu" to clarify the relation between the chemical constituents and the original plants, and have reported the structure elucidation of new alkaloids from several kinds of "Bei-mu".[2-5] In this paper, we describe the isolation and structure elucidation of a new alkaloid, tortifoline (**1**), from *Fritillaria tortifolia* (托里貝母), which was harvested in Xinjiang-Uygur province, China.

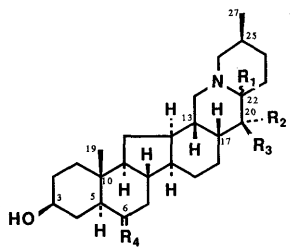
Results and Discussion

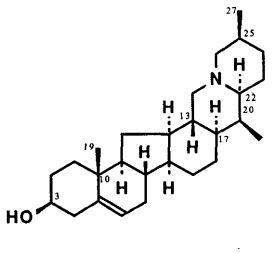
The dried bulbs of *F. tortifolia* were extracted with 50% aqueous acetone, and the alkaloid-containing fraction was hydrolyzed with 1 N methanolic-HCl. The hydrolyzate was chromatographied over silica gel and a new alkaloid, tortifoline (**1**), was obtained as white cubes from MeOH along with the known alkaloids, solanidine and imperialine.

The mass spectrum (MS) of **1** revealed the M^+ ion peak at *m/z* 415 and the molecular formula was determined as $C_{27}H_{45}NO_2$ by high-resolution MS. The base peak was observed at *m/z* 111, which is a typical fragment ion of 5α-cevanine alkaloids without the hydroxyl group at C-20.[6] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed three methyl signals at δ0.80 (d, J=5.1 Hz), δ0.82 (d, J=6.2 Hz), and δ0.98 (s), and two methine protons bearing a hydroxyl group at δ3.66 ($W_{1/2}$=22 Hz), 3.87 ($W_{1/2}$=8 Hz). The chemical shifts and the half widths of these hydroxyl methine protons implied the presence of 3β- and 6β-hydroxyl groups. The presence of the 6β-hydroxyl group was supported by the chemical shift of the Me_{19} group which was observed at lower field than that of 5α-cholestanol due to 1,3-diaxial interaction. Moreover, the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** revealed signals due to A, B and C ring carbons corresponding to those of delavine (**2**), 20-deoxy-5α-cevanine-3β,6β-diol (see Table I).

In the ^{13}C-NMR spectrum of **1** (Table I), the signals due to the F ring carbons resemble those of chuanbeinone (**3**), except for that of C-22, which appeared at 2 ppm lower field than that of **3** (δ68.8 for **1**, and 66.9 for **3**, see Table I). This implies that **1** has the same configurations as **3** at C-25 (25*S*), but the D/E juncture and/or configuration of C-22 and/or C-20 are different from those of **3** (20*R*, 22*R*, 25*S*, D/E *cis*).

Two secondary methyl signals were assigned by ^{1}H–^{13}C correlated spectroscopy (COSY). The signal of the Me_{27} protons were observed at δ 0.82 (δ19.7 in the ^{13}C-NMR). This revealed that there was no 1,3-diaxial interaction involv-





shinonomenine (**4**)

		R_1	R_2	R_3	R_4
tortifoline	(1)	β-H	H	Me	OH, H
delavine	(2)	α-H	Me	H	OH, H
chuanbeinone	(3)	β-H	Me	H	=O

Fig. 1

Table I. ^{13}C-NMR Data ($CDCl_3$, 22.5 MHz)

Carbon No.	Tortifoline (1)	Delavine (2)	Chuanbeinone (3)
1	39.0	39.4	37.6
2	31.4	31.4	30.6
3	72.0	71.9	70.9
4	34.9	34.8	30.3
5	48.2	48.1	56.4
6	73.0	73.2	211.1
7	39.3	39.6	46.8
8	36.6	36.7	43.3
9	57.6	57.9	54.8
10	35.4	35.5	38.2
11	30.1	30.3	32.0
12	39.5	39.1	38.2
13	34.7	39.1	37.7
14	40.6	41.2	36.6
15	26.2	28.7	24.4
16	21.4	17.7	24.8
17	44.3	41.6	48.0
18	65.3	59.2	65.7
19	15.1	15.7	12.4
20	37.2	38.9	37.4
21	14.9	14.7	11.4
22	68.8	62.5	66.9
23	30.9	25.0	30.1
24	33.6	30.3	33.6
25	30.7	28.4	31.1
26	59.3	61.7	59.9
27	19.8	18.3	19.8

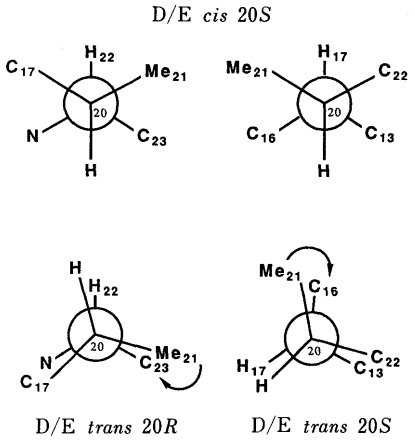


Fig. 2. Newman Projections of Possible Configurations of **1**

Each case has 22*R*, 25*S* configuration. Because a known alkaloid, chuanbeinone (**3**), has D/E *cis* 20*R* configuration, it is excluded.

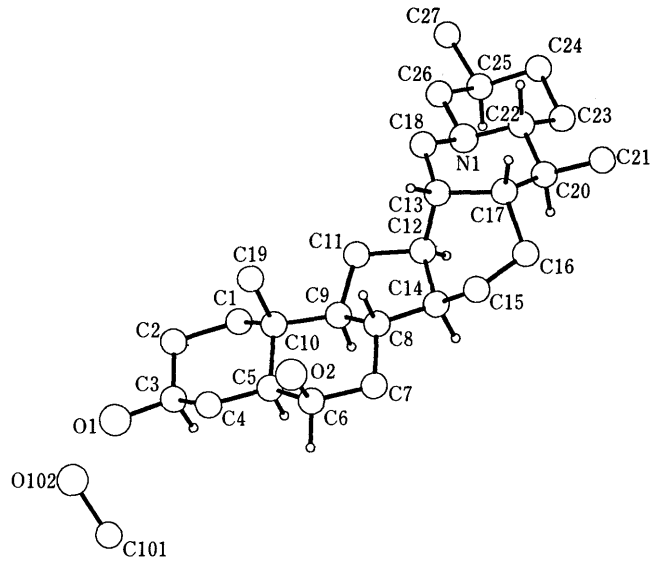


Fig. 3. Perspective Drawing of the Molecule Showing the Relative Configuration of Tortifoline

ing lone pair of nitrogen and Me_{27}, so **1** has the *R* configuration at C-22. The chemical shift of another secondary methyl (Me_{21}, δ_H 0.80, δ_C 15.0) also indicated no 1,3-diaxial interaction or overlapping effect with C-16 or C-23 carbon. Our previously reported observation on the relation of ^1H and ^{13}C-NMR chemical shifts to D/E juncture (**2**),[2] Me_{21} (**4**),[7] and H_{22} (**3**),[3] and Dreiding model studies revealed that the combination of 20*S*,22*R*,25*S* D/E *cis* configuration was suitable to explain the NMR data (Fig. 2). Therefore the structure of **1** was assumed to be (20*S*, 22*R*, 25*S*) D/E *cis* 5α-cevanine-3β,6β-diol, and this was confirmed by an X-ray crystal structure elucidation (Fig. 3 and ref. 8).

The relative stereochemistry of **1** was deduced on the basis of the β-configuration of the C-10 methyl group as shown in Fig. 3. The results showed that all rings are in a chair conformation. The ring fusions are as follows: A/B *trans*, B/C *trans*, C/D *cis*, D/E *cis*. The configurations at the chiral centers are as follows: 3-OH β-equatorial, 6-OH β-axial, Me_{19} β-axial, Me_{21} β-equatorial, H_{22} β-axial, Me_{27} β-equatorial, and the lone pair of nitrogen is α-axial.

Tortifoline (**1**) is the second 5α-cevanine alkaloid with an unusual (22*R*)-*trans*-quinolizidine moiety to be isolated from natural sources. Chuanbeinone (**3**) and tortifoline (**1**) are interesting compounds in relation to C-nor D-homo steroidal alkaloid biosynthesis.

TABLE II. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Temperature Factors ($Å^2 \times 10$) with e.s.d. Values in Parentheses for Tortifoline

	x	*y*	*z*	B_{eq}
C(1)	1226 (9)	1301 (15)	3130 (7)	74 (3)
C(2)	762 (14)	249 (19)	2064 (10)	107 (6)
C(3)	−310 (16)	672 (31)	1659 (13)	168 (9)
C(4)	−490 (9)	2415 (29)	1358 (10)	129 (8)
C(5)	−83 (6)	3309 (19)	2432 (6)	77 (4)
C(6)	−358 (7)	4992 (23)	2348 (7)	95 (5)
C(7)	27 (7)	5899 (19)	3442 (7)	80 (4)
C(8)	1175 (5)	5581 (11)	3856 (5)	45 (2)
C(9)	1388 (5)	3882 (12)	4026 (5)	49 (2)
C(10)	1103 (6)	2981 (12)	2887 (5)	49 (2)
C(11)	2540 (8)	3832 (15)	4667 (8)	79 (3)
C(12)	2678 (6)	5254	5476 (5)	47 (2)
C(13)	3668 (8)	6061 (16)	5393 (7)	71 (3)
C(14)	1656 (7)	6226 (14)	5056 (7)	64 (3)
C(15)	1869 (9)	7935 (15)	4963 (10)	81 (4)
C(16)	2612 (13)	8465 (15)	5992 (14)	98 (5)
C(17)	3735 (10)	7674 (15)	6077 (10)	83 (4)
C(18)	4587 (6)	5126 (15)	5757 (7)	68 (3)
C(19)	1798 (5)	3434 (12)	2006 (6)	49 (2)
C(20)	4203 (9)	7428 (15)	7370 (9)	75 (3)
C(21)	4482 (17)	9019 (21)	7851 (19)	125 (7)
C(22)	5134 (7)	6325 (14)	7619 (8)	68 (3)
C(23)	5394 (12)	6140 (22)	8872 (11)	105 (6)
C(24)	6345 (12)	4927 (21)	9184 (12)	99 (5)
C(25)	5986 (11)	3446 (22)	8603 (12)	99 (5)
C(26)	5685 (7)	3765 (16)	7252 (10)	76 (3)
C(27)	6930 (13)	2219 (29)	8676 (16)	132 (8)
N(1)	4857 (4)	4910 (11)	7029 (5)	52 (2)
O(1)	−785 (13)	−174 (23)	629 (9)	199 (9)
O(2)	−28 (8)	5674 (17)	1350 (6)	115 (4)
C(101)	−2637 (16)	−2078 (23)	1605 (17)	123 (7)
O(102)	−1743 (12)	−2475 (18)	1002 (12)	132 (6)

e.s.d., estimated standard deviation. C(101) and O(102) represent the atoms of MeOH. $B_{eq} = 4/3 \sum_i \sum_j \beta_{ij} \boldsymbol{a}_i \boldsymbol{a}_j$.

TABLE III. Bond Lengths (Å) with e.s.d. Values in Parentheses for Tortifoline

C(1)–C(2)	1.58 (2)	C(1)–C(10)	1.51 (2)
C(2)–C(3)	1.45 (3)	C(3)–C(4)	1.58 (4)
C(3)–O(1)	1.46 (3)	C(4)–C(5)	1.50 (3)
C(5)–C(6)	1.52 (3)	C(5)–C(10)	1.58 (2)
C(6)–C(7)	1.51 (3)	C(6)–O(2)	1.45 (3)
C(7)–C(8)	1.52 (2)	C(8)–C(9)	1.53 (1)
C(8)–C(14)	1.54 (2)	C(9)–C(10)	1.54 (2)
C(9)–C(11)	1.56 (2)	C(10)–C(19)	1.55 (2)
C(11)–C(12)	1.56 (2)	C(12)–C(13)	1.50 (2)
C(12)–C(14)	1.59 (2)	C(13)–C(17)	1.62 (2)
C(13)–C(18)	1.46 (2)	C(14)–C(15)	1.54 (2)
C(15)–C(16)	1.48 (2)	C(16)–C(17)	1.62 (2)
C(17)–C(20)	1.54 (2)	C(18)–N(1)	1.48 (2)
C(20)–C(21)	1.53 (3)	C(20)–C(22)	1.55 (2)
C(22)–C(23)	1.45 (2)	C(22)–N(1)	1.44 (2)
C(23)–C(24)	1.63 (3)	C(24)–C(25)	1.50 (3)
C(25)–C(26)	1.58 (2)	C(25)–C(27)	1.64 (3)
C(26)–N(1)	1.47 (2)	C(101)–O(102)	1.52 (3)

Experimental

Melting points were determined on a Leitz Wetzlar micro melting point

TABLE IV. Bond Angles (°) with e.s.d. Values in Parentheses for Tortifoline

C(2)–C(1)–C(10)	114 (1)	C(1)–C(2)–C(3)	108 (2)
C(2)–C(3)–C(4)	114 (2)	C(2)–C(3)–O(1)	112 (2)
C(4)–C(3)–O(1)	107 (2)	C(3)–C(4)–C(5)	108 (2)
C(4)–C(5)–C(6)	114 (2)	C(4)–C(5)–C(10)	111 (2)
C(6)–C(5)–C(10)	114 (1)	C(5)–C(6)–C(7)	115 (2)
C(5)–C(6)–O(2)	111 (2)	C(7)–C(6)–O(2)	111 (2)
C(6)–C(7)–C(8)	109 (1)	C(7)–C(8)–C(9)	112 (1)
C(7)–C(8)–C(14)	116 (1)	C(9)–C(8)–C(14)	102 (1)
C(8)–C(9)–C(10)	113 (1)	C(8)–C(9)–C(11)	103 (1)
C(10)–C(9)–C(11)	117 (1)	C(1)–C(10)–C(5)	108 (1)
C(1)–C(10)–C(9)	110 (1)	C(1)–C(10)–C(19)	109 (1)
C(5)–C(10)–C(9)	105 (1)	C(5)–C(10)–C(19)	113 (1)
C(9)–C(10)–C(19)	111 (1)	C(9)–C(11)–C(12)	105 (1)
C(11)–C(12)–C(13)	110 (1)	C(11)–C(12)–C(14)	104 (1)
C(13)–C(12)–C(14)	115 (1)	C(12)–C(13)–C(17)	110 (1)
C(12)–C(13)–C(18)	113 (1)	C(17)–C(13)–C(18)	113 (1)
C(8)–C(14)–C(12)	105 (1)	C(8)–C(14)–C(15)	110 (1)
C(12)–C(14)–C(15)	113 (1)	C(14)–C(15)–C(16)	111 (1)
C(15)–C(16)–C(17)	112 (1)	C(13)–C(17)–C(16)	112 (1)
C(13)–C(17)–C(20)	110 (1)	C(16)–C(17)–C(20)	108 (1)
C(13)–C(18)–N(1)	113 (1)	C(17)–C(20)–C(21)	105 (1)
C(17)–C(20)–C(22)	116 (1)	C(21)–C(20)–C(22)	112 (1)
C(20)–C(22)–C(23)	101 (1)	C(20)–C(22)–N(1)	109 (1)
C(23)–C(22)–N(1)	112 (1)	C(22)–C(23)–C(24)	109 (1)
C(23)–C(24)–C(25)	108 (2)	C(24)–C(25)–C(26)	107 (1)
C(24)–C(25)–C(27)	112 (2)	C(26)–C(25)–C(27)	102 (2)
C(25)–C(26)–N(1)	110 (1)	C(18)–N(1)–C(22)	111 (1)
C(18)–N(1)–C(26)	107 (1)	C(22)–N(1)–C(26)	113 (1)

TABLE V. Anisotropic Thermal Parameters ($\times 10^4$) with e.s.d. Values in Parentheses for Tortifoline

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	160 (9)	213 (15)	91 (6)	−39 (10)	20 (5)	−17 (8)
C(2)	243 (17)	311 (24)	132 (10)	−149 (17)	76 (10)	−62 (13)
C(3)	270 (22)	774 (66)	197 (17)	−340 (35)	132 (16)	−253 (29)
C(4)	99 (7)	784 (68)	146 (11)	−35 (18)	16 (6)	−184 (24)
C(5)	84 (5)	431 (26)	81 (6)	−52 (10)	20 (4)	−49 (10)
C(6)	96 (6)	555 (40)	97 (7)	94 (14)	27 (5)	4 (14)
C(7)	101 (7)	393 (26)	102 (7)	38 (11)	28 (5)	−21 (11)
C(8)	76 (4)	136 (9)	83 (5)	0 (5)	18 (3)	3 (5)
C(9)	80 (4)	172 (10)	71 (4)	−1 (6)	−4 (3)	16 (6)
C(10)	86 (4)	177 (11)	66 (4)	−26 (6)	15 (3)	7 (5)
C(11)	130 (8)	234 (17)	126 (8)	48 (10)	−22 (6)	−55 (10)
C(12)	97 (5)	107 (7)	75 (4)	8 (5)	2 (3)	−18 (5)
C(13)	111 (7)	298 (20)	99 (6)	−8 (9)	51 (5)	2 (10)
C(14)	101 (6)	221 (14)	115 (7)	8 (7)	33 (5)	−43 (9)
C(15)	128 (9)	197 (15)	178 (12)	41 (9)	17 (7)	−5 (11)
C(16)	187 (13)	140 (12)	220 (15)	36 (11)	0 (10)	1 (12)
C(17)	145 (9)	202 (16)	162 (11)	−43 (10)	14 (7)	29 (11)
C(18)	83 (5)	296 (19)	111 (7)	−27 (8)	23 (4)	−61 (10)
C(19)	74 (4)	171 (10)	87 (5)	11 (5)	15 (3)	25 (6)
C(20)	123 (8)	207 (14)	146 (9)	−37 (9)	13 (6)	−33 (9)
C(21)	210 (18)	244 (22)	303 (25)	−11 (17)	46 (16)	−64 (22)
C(22)	94 (6)	232 (16)	130 (8)	−35 (8)	20 (5)	−40 (9)
C(23)	166 (13)	371 (30)	150 (11)	−2 (16)	−6 (8)	−78 (17)
C(24)	158 (11)	329 (27)	162 (11)	38 (15)	10 (8)	−44 (16)
C(25)	142 (10)	340 (26)	169 (12)	−5 (15)	−7 (8)	−40 (16)
C(26)	90 (6)	247 (19)	162 (10)	4 (9)	−6 (5)	−23 (11)
C(27)	147 (12)	523 (54)	233 (19)	85 (21)	−19 (11)	81 (28)
N(1)	59 (3)	205 (10)	97 (4)	−5 (5)	6 (2)	−20 (6)
O(1)	338 (20)	932 (61)	198 (12)	−438 (32)	147 (13)	−274 (23)
O(2)	191 (9)	539 (27)	102 (5)	191 (13)	48 (5)	75 (9)
C(101)	202 (17)	350 (32)	241 (20)	−80 (20)	53 (14)	−50 (22)
O(102)	203 (12)	354 (22)	302 (18)	35 (14)	81 (11)	29 (18)

The temperature factor is of the form;
$\exp[-(\beta_{11}h_2^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)]$.

apparatus and are uncorrected. Optical rotations were measured with a JASCO A-102 spectrometer. ^{1}H-NMR spectra were run on a JEOL JNM FX-100 (100 MHz) or GX-270 (270 MHz) spectrometer in $CDCl_3$ solution and ^{13}C-NMR spectra on a JEOL FX-90Q (22.5 MHz) or GX-270 (67.5 MHz) spectrometer in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. Electron impact mass spectrometry (EI-MS) was done with a JEOL JMS-D-300 machine. Thin layer chromatography (TLC) was performed on Merck precoated plates, Kieselgel 60 F_{254}. Column chromatography was carried out on Wakogel C-300 (300 mesh).

Isolation Dried bulbs of *F. tortifolia* (2.9 kg) were powdered and extracted four times with 4 l of acetone–water (1 : 1). After concentration, the extract was washed with hexane to remove fatty substances (13.0 g), and then applied to a column (8.5 × 23 cm) of Diaion HP-20 (Mitsubishi Kasei Co., Ltd). The column was eluted with H_2O–methanol using increasing MeOH concentration. The alkaloid-containing fractions (50—100% MeOH) were combined and concentrated (32.8 g). The combined alkaloid-containing fraction (32.8 g) was hydrolyzed with 1 N methanolic-HCl (250 ml) for 6 h, and worked up to afford 2.50 g of crude alkaloid. The crude alkaloid was fractionated by repeated silica gel column chromatography using the solvent systems of hexane–EtOH–Et_2NH (12 : 1 : 1) and cyclohexane–EtOAc–MeOH (2 : 2 : 1). Tortifoline (**1**, 43.0 mg) was obtained along with solanidine (162.1 mg), imperialine (101.1 mg), and delavinone (73.5 mg).

Tortifoline (1) White cubes from MeOH. mp 206—208 °C, $[\alpha]_D$ −41.4° (c = 1.07, $CHCl_3$). $C_{27}H_{45}NO_2$ (obsd. 415.34327, calcd. 415.344981). MS m/z: 415 (M^+), 400 (M^+-CH_3), 358, 164, 111 (base peak), 98. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 2720 (*trans*-quinolizidine).[9] ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.80 (3H, d, J = 5.1 Hz, 21-H), 0.82 (3H, d, J = 6.2 Hz, 27-H), 0.98 (3H, s, 19-H), 3.66 (1H, m, $W_{1/2}$ = 22 Hz, 3α-H), 3.87 (1H, m, $W_{1/2}$ = 8 Hz, 6α-H). ^{13}C-NMR (22.5 MHz, $CDCl_3$): see Table I.

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8) The crystal of **1** is monoclinic, $C_{27}H_{45}NO_2 \cdot CH_3OH$, M_1 = 447.7, space group $P2_1$, and the cell dimensions are, a = 13.150 (1), b = 8.797 (1), c = 11.701 (2) Å, β = 100.74 (1)°, D_c = 1.118 g cm^{-3}, Z = 2, CuK_α radiation (λ = 1.54178 Å), ν = 5.6 cm^{-1}. Crystals were obtained as a methanol solvate. In total, 2431 unique reflections within $2\theta \leq 130°$ were measured on a Rigaku AFC-5R diffractometer using the ω–2θ scan technique. The structure was solved by direct methods and refined anisotropically by a block-diagonal least-squares method to R = 0.147 for 1942 observed reflections with $|F_O| > 3\sigma(F_O)$, the large R value probably being due to poor quality of the crystal used. H atoms could not be located. Bond lengths (σ = 0.01—0.04 Å) and bond angles (1—2°) were of limited accuracy, but did not deviate greatly from the values expected on chemical grounds.
9) F. Bohlmann, *Chem. Ber.*, **91**, 2157 (1958).