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REASSIGNMENT OF THE STEREOCHEMISTRY OF OBLONGININE

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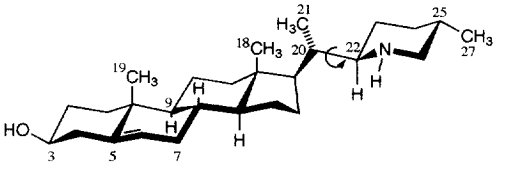
Key Word Index—*Veratrum oblongum*; Oblonginine; steroidal alkaloid.

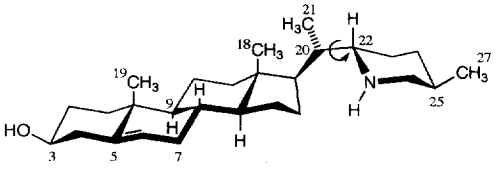
Abstract—The stereochemistry of the steroidal alkaloid oblonginine from *Veratrum oblongum* (Chinese name Changgenglilu), previously reported to possess the structure (22*R*,25*S*)-22,26-epiminocholest-5-en-3β-ol, was re-examined using X-ray crystallography and high resolution NMR spectroscopy and reassigned with regard to C-22 and C-25. Therefore, oblonginine has the structure (22*S*,25*R*)-22,26-epiminocholest-5-en-3β-ol.

INTRODUCTION

In a previous study [1] into the principles of bioactive constituents of *Veratrum oblongum* (Chinese name Changgenglilu), a widely used medicinal herb indigenous to China for treatment of aphasia, a novel alkaloid oblonginine was isolated and characterized along with a group of known compounds. Oblonginine was shown by Kadota *et al.* [1] to be a piperidinyl steroidal alkaloid [2–4], and its configuration was assigned as (22*R*,25*S*)-22,26-epiminocholest-5-en-3β-ol (**1**), the same as a compound derived from solanidine [5]. This was mainly on the basis of NMR data obtained at 400 MHz and a comparison of this data with the NMR data of compounds with similar structures in the literature [6]. We have obtained good quality single crystals of oblonginine (**2**) and have carried out single crystal X-ray diffraction experiments which unambiguously give the relative configurations of all the asymmetric centres. We now report the reassignment of the configuration of the asymmetric centres at C-22 and C-25 and confirm by NMR spectroscopy that our sample of oblonginine (**2**) is identical with that obtained by Kadota *et al.* [1].



1: 22R, 25S



2: 22S, 25R

RESULTS AND DISCUSSION

After purification, using column chromatography, deep red crystals of oblonginine monohydrate were obtained by repeated crystallization from a mixture of acetone and hexane. The solid state structure of **2** was confirmed by single crystal X-ray diffraction experiments and is depicted with the numbering scheme in Fig. 1.

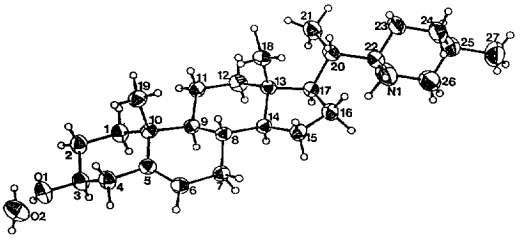


Fig. 1. ORTEP view [11] of oblonginine (**2**) showing the numbering scheme. Displacement ellipsoids are shown at the 50% probability level.

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Oblonginine crystallises in the monoclinic space group $P2_1$ with the two molecules in the unit cell being firmly hydrogen bonded by the two molecules of water. In these interactions, the water acts as both a donor (O2...O1, 2.849 Å and O2...N1, 2.930 Å by the symmetry operations $-x$, $1/2+y$, $2-z$ and $-x$, $1/2+y$, $1-z$ respectively) and as an acceptor (O1...O2, 2.788 Å by the symmetry operation x, y, z) thus producing a stable crystalline structure from an otherwise largely carbon skeleton.

However, the relative stereochemistry of the steroid framework and the piperidine ring is of particular importance and this had been previously assigned [1] as 22*R*, 25*S*, from NMR data obtained at 400 MHz. The solution and refinement of our crystallographic data, using the SHELX suite of programs (SHELXS and SHELXL-93) according to the procedure of Flack [7] shows without doubt that the alternative configuration of 22*S*, 25*R* is correct.

In order to confirm that the compound isolated and purified in the present study was indeed identical to that isolated by Kadota *et al.* [1], our compound was examined by NMR spectroscopy. The ^{1}H NMR and ^{13}C NMR spectral data of our sample of oblonginine (**2**) are given in Table 1 together with those reported by Kadota [1] for comparison. In addition to the one dimensional (1D) ^{1}H and ^{13}C NMR spectra, two dimensional DQFCOSY, TOCSY, NOESY and HMQC spectra were obtained. The HMQC spectrum allowed the unambiguous identification of all the geminal proton pairs. Concerted use of this data, together with the proton correlations shown in the DQFCOSY and TOCSY spectra, allowed the complete assignment of all the proton and carbon-13 resonances. The location, axial or equatorial, of all protons has been determined for the first time, and all of the data are consistent with the structure obtained from the X-ray study.

There was excellent agreement (within 0.1 ppm allowing for a possible reference error of 0.1 ppm) between the carbon-13 chemical shifts of our sample and that of Kadota *et al.* [1] and the assignments concur. With the exception of H-2 (axial), which lies in a very crowded region of the spectrum, the agreement between the proton shifts was also very good (within 0.02 ppm) and the assignments again concur. Thus, our sample was identical with that obtained by Kadota [1]. The NOESY spectrum revealed all the expected NOEs between geminal proton pairs; NOEs between the 18/19 and 18/21 methyl groups; most of the expected NOEs between 1, 3 diaxial proton pairs; between methyl groups and nearby (but not *J*-coupled) protons; and the expected NOEs between non-axial vicinal proton pairs, except those which are too close to the diagonal such as the 11-protons. Several other NOEs were identified, viz: H-4eq/H-6, H-7eq/15α, H-16α/H-22, H-16β/H-22, H-17/H-23eq. A cross peak assigned to H-21/H-23ax was also observable in the NOESY spectrum. All these are consistent with the crystal structure.

The original proposal [1] of 22*R*-stereochemistry for oblonginine rested only on the observation of an NOE between H-21 and H-23eq. Since rotation of the piperidine ring about the C-20–C-22 bond in **2** allows H-23eq in the 22*S*-molecule to get equally close to H-21, the two configurations cannot be distinguished in this way. Moreover it would be almost impossible to say which multiplet was enhanced in a 1-D experiment in which the H-21 doublet was 'selectively' irradiated, as the shift of H-20 (δ 1.502) is almost the same as that of H-23eq (δ 1.495). In conclusion, the configurations at C-22 and C-25 in oblonginine are unambiguously defined as 22*S* and 25*R* as shown in the formula **2**. Though the occurrences of steroidal alkaloids with a 25*R*-configuration in plants of the *Veratrum* are very seldom, these compounds are observed in other plants [3, 4]. A compound possessing the structure (22*S*,25*R*)-22,26-epiminocholest-5-en-3β-ol, now assigned as oblonginine, has been reported by Havel and Cerny [8], and was obtained by the degradation of solasodine. The selective ^{1}H NMR data reported are identical with those obtained for oblonginine, with the exception of H_3-27, for which we are confident that the newly collected 600 MHz data are correct. The discrepancies in melting points could be due to the nature of the crystals, since in our studies, all solid state analysis was performed on the monohydrate of oblonginine as shown in the crystal structure. The difference in $[\alpha]_D$ values is not so easily explained but their value of $+19^\circ$ appears very much out of line with the rest of the compounds they have analysed, and we can only assume that this was a mistake.

EXPERIMENTAL

Crystallographic studies. Crystal data for **2**, $C_{27}H_{45}NO\,H_2O$, $M = 417.66$, monoclinic, space group $P2_1$, $a = 7.971(1)$, $b = 9.819(1)$, $c = 16.477(1)$ Å, $\beta = 101.14(1)^\circ$, $U = 1265.3(2)$ Å^3, $Z = 4$, $D_c = 1.096$ Mg M^{-3}, $F(000) = 464$, λ(Cu-$K\alpha$) = 1.54180 Å. $\mu = 0.512$ mm^{-1}. A plate $0.7 \times 0.7 \times 0.3$ mm was mounted on an Enraf-Nonius CAD4 four circle diffractometer, 2812 unique reflections were collected by ω-2θ scans for $2.73^\circ \leqslant \theta \leqslant 76.36^\circ$ and phased by direct methods [9]. Refinement [10] was by full-matrix least squares on F^2 with anisotropic thermal parameters for non-hydrogen atoms and all hydrogen atom positions determined from a difference Fourier synthesis. At convergence, $R = 0.039$, $R_w = 0.107$ and GOOF = 0.969 for 2742 observed reflections [$I > 2\sigma(I)$]. Atomic coordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, U.K.

NMR spectroscopy. The spectra were measured in $CDCl_3$ using a Varian INOVA 600 spectrometer operating at 599.9 MHz for protons and 150.9 MHz for ^{13}C nuclei. Exact proton chemical shifts were determined from the 1D proton spectrum and in regions of severe overlap, from the 2D DQFCOSY spectrum.

Table 1. 600 MHz ^{1}H NMR and 150 MHz ^{13}C NMR data of oblonginine (**2**) in $CDCl_3$

δ_C		C		δ_H	J (Hz)
37.1	[37.3]*	1	ax	1.061† [1.05] *m*	
			eq	1.834 [1.85] *m*	
31.6	[31.7]	2	ax	1.489 [1.55] *m*	
			eq	1.825 [1.82] *m*	
71.7	[71.7]	3	ax	3.513 [3.51] *tdd*	11.2, 11.2, 4.7, 4.5 [*tdd* 11.5, 11.5, 5.5, 4.0]
42.2	[42.2]	4	ax	2.223 [2.22] *ddddd*	13.1, 11.2, 3.0, 3.0, 2.3
			eq	2.288 [2.28] *ddd*	13.1, 5.1, 2.2
140.7	[140.9]	5			
121.5	[121.6]	6		5.340 [5.34] *dt*	5.4, 2.0, 2.0
31.8	[32.0]	7	ax	1.512 [1.50] *m*	
			eq	1.962 [1.96] *dtd*	17.0, 5.1, 5.1, 2.8
31.9	[32.0]	8	ax	1.444 [1.45] *m*	
50.0	[50.2]	9	ax	0.915 [0.93] *m*	
36.4	[36.5]	10			
21.0	[21.1]	11	ax	1.44 [1.49] *m*	
			eq	1.48 [1.49] *m*	
39.8	[39.9]	12	ax	1.149 [1.16] *ddd*	12.9, 12.5, 4.6
			eq	1.992 [2.10] *dt*	12.7, 3.5, 3.5 [*ddd* 12.5, 4.0, 2.4]
42.3	[43.5]	13			
56.5	[56.7]	14	ax	0.979 [0.99] *m*	
24.2	[24.3]	15	β	1.084 [1.08] *m*	
			α	1.576 [1.60] *dddd*	12.0, 10.0, 7.2, 3.3
27.6	[27.8]	16	β	1.336 [1.35] *dddd*	13.2, 11.5, 9.8, 3.3
			α	1.751 [1.74] *m*	
53.0	[53.2]	17	α	1.197 [1.21] *dt*	10.7, 9.3, 9.3
11.6	[11.8]	18	Me	0.682 [0.70] *s*	
19.3	[19.4]	19	Me	0.995 [1.01] *s*	
40.8	[41.0]	20		1.502[1.51] *m*	
13.5	[13.6]	21	Me	0.890[0.90] *d*	6.8, [*d* 6.5]
58.9	[59.1]	22	ax	2.446 [2.45] *dt*	11.4, 2.6, 2.6, [*dt* 11.0, 2.5]
25.1	[25.3]	23	ax	1.103[1.11] *m*	
			eq	1.495 [1.49] *m*	
33.9	[34.0]	24	ax	0.952[0.96] *m*	
			eq	1.786 [1.79] *m*	
32.6	[32.8]	25	ax	1.412 [1.43] *m*	
55.2	[55.3]	26	ax	2.267[2.26] *dd*	12.0, 11.0, [*t* 11.5]
			eq	3.021 [3.02] *ddd*	12.0, 4.0, 2.1 [*ddd* 11.5, 4.0, 2.0]
19.4	[19.6]	27	Me	0.799 [0.81] *d*	6.6

* Data in square brackets are obtained previously at 400 MHz by Kadota *et al.* [1].

† Measurement is possible at 600 MHz at δ 0.001.

Proton coupling constants could only be determined from the 1D proton spectra where the multiplets were sufficiently well resolved.

The DQFCOSY proton spectrum was obtained over a spectral width (SW) of 1950 Hz; 2 K data points; and 220 increments each with 32 transients per FID. The TOCSY proton spectrum was acquired over the same spectral width; 1 K data points; 200 increments each with 48 transients per FID and a mixing time of 60 ms were used. The NOESY spectrum was similarly acquired with a mixing time of 700 ms. The proton detected one-bond ^{1}H-^{13}C correlation (HMQC) spectrum was obtained with ^{13}C broad band decoupling during acquisition of the proton FIDs and with 256 increments each with 32 scans per FID. The parameters used were SW(^{1}H) = 3350 Hz; 2 K data points; SW(^{13}C) = 25 000 Hz. In all cases the data were processed using shifted sine-bell squared functions in both dimensions with zero filling of the F1-data to 1 K data before transformation.

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