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THE CHARACTERIZATION AND ABSOLUTE STEREOCHEMISTRY OF BARBALINE, A DITERPENOID ALKALOID FROM *DELPHINIUM BARBEYI*

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Key Word Index—*Delphinium barbeyi*; Ranunculaceae; diterpenoid alkaloid; barbaline.

Abstract—The structure and absolute stereochemistry of a new diterpenoid alkaloid, barbaline, was determined by NMR spectroscopy techniques and single-crystal X-ray diffraction analysis.

INTRODUCTION

The tall larkspur *Delphinium barbeyi* is a major poisonous plant threat to cattle grazing on rangelands in the Western United States [1]. The plant contains significant amounts of norditerpenoid alkaloids [2] and two of these alkaloids, methyl-lycaconitine and 14-deacetylnudicauline, have been established as potent toxins to cattle [3]. A diterpenoid alkaloid, barbisine (**1**), has also been isolated and characterized from *D. barbeyi* [4]. Barbisine (**1**), in addition to vakognavine (**2**) and 15-deacetylvakognavine (**3**) obtained from *Aconitum palmatum* [5–7], are the only known naturally occurring *N*-C(19)-seco diterpenoid alkaloids which have been described. We now report the isolation, characterization and absolute stereochemistry of a new *N*-C(19)-seco diterpenoid alkaloid, barbaline (**4**), from *D. barbeyi*.

RESULTS AND DISCUSSION

HPLC of residual fractions obtained during the preparative isolation of toxic norditerpenoid alkaloids from *D. barbeyi* for the investigation of structure–activity relationships [8] yielded barbaline (**4**), ($C_{34}H_{37}NO_{11}$, mp 297° (dec.)). The ^{1}H NMR (see Experimental) and ^{13}C NMR (Table 1) of **4** exhibit resonances for an exocyclic methylene (δ_H 4.96, 5.06, δ_C 113.9, 136.8), a tertiary methyl (δ_H 1.16, δ_C 23.3), an *N*-methyl (δ_H 2.43, δ_C 33.6) and a tertiary aldehyde (δ_H 9.69, δ_C 196.4) which are consistent with similar characteristic signals reported for previously described *N*-C(19)-seco diterpenoid alkaloids [4, 6, 7]. The NMR spectra of **4** also establish the presence of three acetates (δ_H 1.96, 2.03, 2.10), a benzoate (δ_H 7.49–8.00) and a keto group (δ_C 206.2). Barbaline (**4**) forms a monoacetate (**5**) in the presence of acetic anhydride/pyridine (δ_H 2.18, 3H; δ_H 5.46, 1H), indicating the presence of a secondary hydroxyl group in the alkaloid.

Vicinal coupling patterns observed between methine protons occurring on carbons substituted with two of the acetates (δ_H 5.22, 5.55) and the benzoate (δ_H 6.09) in the two-dimensional COSY NMR of **4** indicate that these ester substituted carbons constitute an acetate–benzoate–acetate substituted three-carbon fragment. Moreover, the lack of additional couplings of the two acetate methine protons of this fragment to protons other than the proton on the benzoate-substituted carbon indicates that the three- carbon fragment is connected to quaternary carbons at both ends. Long-range C–H correlations obtained in a two-dimensional HETCOR experiment confirm coupling between protons of the tertiary alkyl methyl group (δ_H 1.16) and one of the acetate substituted carbons (δ_C 72.4) and between that acetate-substituted carbon and the methine proton on the other acetate-substituted carbon (δ_H 5.55) in the fragment. Two bond coupling is also observed between the methine proton on the benzoate-substituted carbon (δ_H 6.09) and the acetate-substituted carbons (δ_C 71.9, 72.4). The benzoate-substituted methine proton also exhibits long-range coupling to a quaternary carbon (δ_C 56.5, C-10), consistent with isolation of the three-carbon fragment between two quaternary centres. Long-range coupling is also observed between protons on the tertiary methyl group and a methine carbon (δ_C 57.6). These establish the three-carbon acetate–benzoate–acetate-substituted fragment as carbons 1, 2 and 3 of the diterpenoid alkaloid structure. The long-range coupling data also places the tertiary methyl group on the quaternary C-4 carbon in **4** and defines C-5 as a

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Table1. Chemical shifts* of ^{13}C resonances for barbaline (**4**) and barbaline acetate (**5**)

C	4	5
C-1	72.4	72.3
C-2	66.6	66.6
C-3	71.9	71.8
C-4	49.2	49.8
C-5	57.6	58.4
C-6	62.7	60.1
C-7	67.6	69.1
C-8	49.5	48.4
C-9	48.6	49.2
C-10	56.5	56.6
C-11	71.0	70.7
C-12	60.0	59.8
C-13	206.2	205.3
C-14	53.8	54.1
C-15	30.1	29.8
C-16	136.8	136.0
C-17	113.9	114.3
C-18	23.3	23.5
C-19	196.4	195.0
C-20	66.0	66.0
$\underline{C}H_3N$	33.6	33.6
Bz C-1	129.2	129.1
Bz C-2	129.8	129.8
Bz C-3	128.8	128.8
Bz C-4	133.7	133.7
Bz C-5	128.8	128.8
Bz C-6	129.8	129.8
Bz $\underline{C}OO$	164.9	164.9
$\underline{C}H_3COO$ (1)	20.9[a]	20.9[a]
$\underline{C}H_3COO$ (3)	20.6	20.6
$\underline{C}H_3COO$ (7)		20.3
$\underline{C}G_3COO$ (11)	21.5[a]	21.0[a]
$CH_3\underline{C}OO$ (1)	169.2[b]	169.1[b]
$CH_3\underline{C}OO$ (3)	170.2	170.2
$CH_3\underline{C}OO$ (7)		169.6
$CH_3\underline{C}OO$ (11)	170.6[b]	170.6[b]

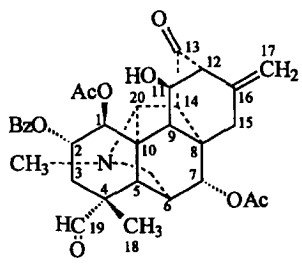
*δ in ppm ($CDCl_3$).
[a,b]May be reversed.

tertiary centre. Based upon comparison of NMR data from **4** with **1**, **2**, and **3** and biogenetic rationale, the aldehyde group was also placed on C-4.

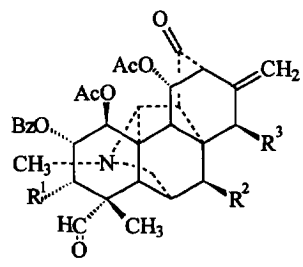
The results of the two-dimensional HETCOR experiment also show long-range coupling between the quaternary carbon attached to C-1 (C-10) and methine protons at two other tertiary carbons (δ_C 53.8, 62.7). One bond and long-range $^1H-^{13}C$ couplings establish the δ_C 62.7 carbon and a carbon at δ_C 66.0 to be attached to the methyl alkaloid nitrogen. The two-dimensional COSY NMR shows a weak coupling between the methine protons on the δ 53.8 and δ 66.0 carbons. The long-range coupling of the proton on the carbon at δ 62.7 to the quarternary carbon (C-10) and the attachment of the δ 62.7 carbon to the nitrogen is consistent with the designation of the tertiary carbon at δ 57.6 as C-5, in accord with similar designations for **1**, **2**, and **3**. These NMR data are consistent with the presence of the C-5, C-6, $N(CH_3)$, C-20, C-10 ring system in **4** with the attachment of a tertiary carbon (C-14) to C-20.

The proton at C-5 displays no vicinal coupling to the proton on C-6. Molecular models show that if the proton at C-5 is in an axial position a dihedral angle approaching 90° exists between it and the proton at C-6, thereby explaining the lack of vicinal coupling between these protons. While the proton on C-6 in **4** shows no coupling to the C-5 proton, it does show coupling to a lowfield proton at δ_H 3.94 which is shifted to δ_H 5.12 upon acetylation of **4** to **5**. This evidence indicates a secondary hydroxyl at C-7. The lack of additional vicinal coupling of the carbinol proton further suggests that C-7 is adjacent to a quaternary carbon centre. The two-dimensional HETCOR results reveal long-range coupling of the proton on C-7 to tertiary carbon C-9 (δ_C 48.6).

Proton and carbon NMR resonances establish the presence of an exocyclic methylene group in **4**. Two-dimensional COSY and HETCOR experiments place the exocyclic methylene carbons at C-16, C-17 in accord with previous observations for similar functionality in barbisine (**1**) [4] and the other *N*-C(19)-seco hetisane-type diterpenoid alkaloids [5–7]. The C-17 methylene protons exhibit long-range coupling to an alkyl methylene carbon (δ_C 30.1) and to a tertiary carbon (δ_C 60.0) whose methine proton (δ_H 2.84), in turn, shows two-bond coupling to a carbonyl carbon (δ_C 206.2) and an acetate-substituted carbon (δ_C 71.0).



Barbisine (**1**)



	R^1	R^2	R^3
Vakognavine (**2**)	H	H	OAc
15-Deacetylvakognavine (**3**)	H	H	OH
Barbaline (**4**)	OAc	OH	H
7-Acetylbarbaline	OAc	OAc	H

These data show that C-15 is a methylene and that C-12 is a methine (δ_C 60.0, δ_H 2.84). An acetate and a carbonyl are located at C-11 or C-13.

The protons on the C-15 methylene show two-bond coupling to a quaternary carbon (δ_C 49.5) and long-range coupling to the carbon (δ_C 48.6) that is coupled to the proton on C-7. The quaternary carbon is also coupled to the proton on C-20. These correlations establish the quaternary carbon (C-8) between C-7 and C-15 and C-9 and further confirm, by default, the placement of the aldehyde at C-4. With the exception of placement of the acetate and keto groups (C-13 or C-11) and designation of the stereochemistry, the proton and carbon NMR data define (**4**) as the basic *N*-C(19)-seco hetisane-type structure of barbaline.

The two-dimensional NOESY spectrum and the CD spectrum of **4** provide data to determine the substitution of C-11 and C-13 (acetate versus keto group) and to determine the relative stereochemistry of the diterpenoid alkaloid. The two-dimensional NOESY spectrum of **4** reveals cross-peak signals for H-3 and H-5. In turn, cross-peaks are observed between each of these protons and the protons of the methyl group at C-4. These data and observed cross-peaks appearing between H-1 and H-20 define a chair conformation for the C-1, C-2, C-3, C-4, C-5 and C-10 ring of **4**, and axial substitution for the protons at C-3 and C-5. The axial configuration of the proton at C-3, coupled with the small vicinal coupling constants (*J* 4.2, 4.2 and 3.9 Hz) and observed NOESY correlations between H-1, H-2, and H-3 are consistent with an H-1_α, H-2_β, H-3_β relative configuration at carbons 1, 2 and 3 in **4**. Observed NOESY correlation between H-5 and H-9, H-9 and H-11, H-9 and H-15a establish 1,3 diaxial relationships between these proton pairs. The lack of correlation of H-6 and H-7 with either H-5 or H-9 establishes the relative configuration of the proton at C-7 to be α. The low field occurrence of H-11 (δ 5.43) and its NOESY corelation to H-9 is consistent with the placement of the third acetate at C-11 (equatorial). The lack of correlation of H-5 and H-9 to the proton at C-7 further dictates the hydroxyl at C-7 to be in the axial position. By default, the two-dimensional NOESY spectral data designates the remaining keto substituent to occur at C-13. This placement is confirmed by the CD spectrum of barbaline (**4**). Diterpenoid alkaloids with a C-11 keto substitution are observed to display a positive Cotton effect in the 300 nm range while C-13 keto-substituted C_{20} diterpenoid alkaloids display a negative Cotton effect in that spectral region [9–10]. The CD spectrum of barbaline (**4**) displays a negative Cotton effect at 303 and 309 nm, in close agreement with CD measurements for barbisine (**1**) [4], confirming keto functionality at C-13 and acetate substitution at C-11 in **4**.

Single-crystal X-ray analysis confirmed the structural characteristics of barbaline (**4**) determined by NMR (Fig. 1). The X-ray analysis established the absolute configuration of the chiral centers of **4** to be 1*S*, 2*R*, 3*S*, 4*S*, 5*S*, 6*R*, 7*R*, 8*R*, 9*R*, 10*R*, 11*S*, 12*R*, 14*S*, 20*R*. Barbaline was therefore established to be [(1*S*-(1β,2α,3α,4α,7β,11α] - 1,3,11 - tris(acetyloxy) - 2 -

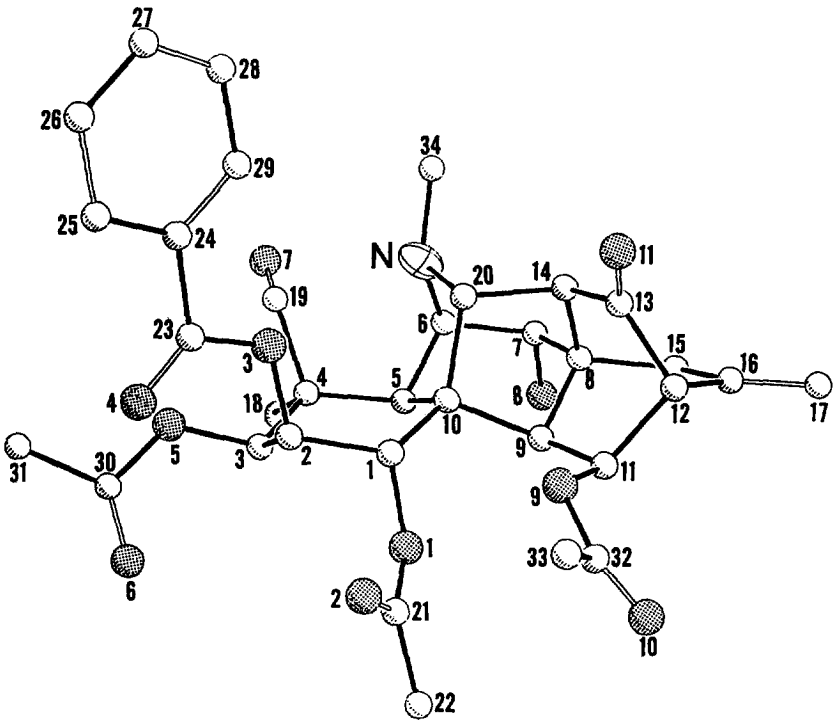


Fig. 1. Perspective view of barbaline with crystallographic numbering scheme. Double bonds are shown unfilled; single bonds, solid; shaded circles represent oxygen atoms.

(benzyloxy)-7-hydroxy-21-methyl-13-oxo-19,21-secohetisan-18-al.

A wide variety of C_{19} norditerpenoid alkaloids present in *D. barbeyi* have been toxicologically evaluated [3]; however, minimal toxicity data is available for C_{20} diterpenoid alkaloids [11] and no toxicological data is available for hetisane-type diterpenoid alkaloids. In the case of compound **4**, evaluation of its mammalian toxicity in a mouse bioassay [8] showed the compound to have an $LD_{50} > 100\ mg\ kg^{-1}$. This high LD_{50} and the low occurrence of barbaline in *D. barbeyi* suggests that this diterpenoid does not play a significant role in the toxicity of the tall larkspurs to cattle.

EXPERIMENTAL

General. Mps uncorr. Prep. open CC (12.5 × 90 cm) was conducted using alumina (60–200 mesh) with $CHCl_3$; 95% aqueous isopropyl alcohol (28:1) as an eluent. HPLC was conducted on an analytical (250 × 4.6 mm) [12] and a semi-prep. (2.0 × 25 cm) column packed with 5 μ basic spherical Al_2O_3 (Phase Separations, Inc.). Column eluent was hexane–95% aqueous isopropyl alcohol (4:1; 17:3). Eluted components were detected by UV absorbance at 220 nm and 280 nm.

NMR spectra were obtained on a Brucker Model AMX 400 spectrophotometer in $CDCl_3$. Carbon/proton multiplicity was determined through a DEPT-135 experiment. Homonuclear and heteronuclear 2D correlation experiments conducted include: ^1H–^1H COSY, ^1H–^1H NOESY, ^1H–^{13}C COSY (carbon observed), long-range ^1H–^{13}C HETCOR (carbon observed) optimized for a long-range heteronuclear coupling of 10 Hz.

Plant material. The aerial portion of *D. barbeyi* (Huth) Huth was collected 3–6 July, 1990, in the Skyline Drive area (elevation 3700 m) of the Manti/LaSalle National Forest, Utah. The plant was in the bud stage. The identity of the plant material was verified at the Intermountain Herbarium, Utah State University, Logan, Utah (Accession No. 316387). The plant material was air dried and ground to pass a 4 mm mesh.

Isolation of barbaline (**4**). Ground *D. barbeyi* plant material (325 kg) was extracted with 95% EtOH (270 l) in a pilot plant scale extractor (60 h). The resulting extract was concd (50 l) in the extractor, removed and concd, *in vacuo*, to a thick syrup in a rotary evaporator. The syrup was poured into stirred 1.5% aq. H_2SO_4 (25 l). The acid solution was divided into two equal portions and each portion was extracted with $CHCl_3$ (3 × 10 l). The $CHCl_3$ extract was discarded. The remaining aqeous acid solns were cooled to 5° with the addition of ice and basified with 20% aqueous NaOH to pH 9. The basified solutions were extracted with $CHCl_3$ (3 × 10 l). The $CHCl_3$ solns were combined and concd, *in vacuo*, on a rotary evaporator to a very thick syrup (620 g). The extract was redissolved in $CHCl_3$ (2000 ml) and a 200 ml aliquot (62 g of extract) was taken for prep. open CC ($CHCl_3$–IPA); 11 frs were collected. Frs 30–33 were concd to dryness, (1.3 g) redissolved in $CHCl_3$ were evaluated on an analytical HPLC column (hexane–95%i-PrOH, 17:3) [12] and frs containing the norditerpenoid alkaloid barbinine were subjected to repetitive semi-prep. HPLC (hexane–95%i-PrOH, 4:1) to obtain barbinine. Frs containing a compound which displayed UV absorbance at 280 nm and eluted from the semi-prep. HPLC column between the norditerpenoid alkaloids methyl-lycaconitine and 14-deacetylnudicauline [12] were collected, concd, redissolved in $CHCl_3$ and chromatographed (semi-prep., hexane–isopropyl alcohol (17:3) to obtain barbaline (**4**) (42 mg). Recrystallization (MeOH) provided pure barbaline (**4**), mp 297°, $[\alpha]_D^{20}$ −17.2° ($CHCl_3$, *c* 1.49). HREIMS $[M-H]^+$: measured, 634.2269, $C_{34}H_{36}NO_{11}$ = 634.2288; M^+ = 607 $[M-28]^+$, 548 $[M-87]^+$, 426, 366, 105 $[C_7H_5O_2]^+$. CD curve (MeOH) $[\theta]_{320}$ −24900 (sh), $[\theta]_{309}$ −359800 (max), $[\theta]_{303}$ −353200 (max); (MeOH + HCl) $[\theta]_{320}$ −75000 (sh), $[\theta]_{308}$ −131000 (max), $[\theta]_{300}$ −127000 (max), $[\theta]_{270}$ −30200 (min).

^1H NMR ($CDCl_3$) δ 9.69 (1H, *br s*, H-19); 7.98 and 7.48–7.66 (5H, each *m*, aromatic protons), 6.09 (1H, *t*, J = 4.2 Hz, H_e-2), 5.55 (1H, *d*, J = 4.2 Hz, H_e-1), 5.43 (1H, *dd*, J = 2.0, 9.5 Hz, H_a-11), 5.22 (1H, *d*, J = 3.9 Hz, H_a-3), 5.06 (1H, *br s*, J = 1.5 Hz, H-17), 4.96 (1H, *br t*, J = 1.5 Hz, H-17), 3.94 (1H, *d*, J = 4.0 Hz, H_e-7), 3.81 (1H, *s*, H-20), 3.03 (1H, *br d*, J = 4.0 Hz, H-6), 2.96 (1H, *dd*, J = 4.0, 9.5, H-9), 2.93 (1H, *dt*, J = 18.0, 1.5 Hz, H-15), 2.84 (1H, *d*, J = 2.0 Hz, H-12), 2.80 (1H, *br d*, J = 4.0 Hz, H-14), 2.52 (1H, *s*, H-5), 2.43 (3H, *s*, H_3C-N), 2.77 (1H, *d*, J = 18.0 Hz, H-15), 2.10 (3H, *s*, H_3C-COO-11), 2.03 (3H, *s*, H_3C-COO-1), 1.96 (3H, *s*, H_3C-COO-3), 1.16 (3H, *s*, H_3C-18). ^1H–^1H NOESY, signal (correlated signal), H-1 (H-2, H-20), H-2 (H-1, H-3), H-3 (H-2, H-5, H_3-C(18)), H-5 (H-3, H-9, H_3-C(18)), H-6 (H_3-CN), H-9 (H-5, H-11, H-15$_a$), H-11 (H-9), H-12 (H-17a), H-14 (H_3-CN), H-15$_a$ (H-9), H-17a (H-12), H-17b (H-15$_e$), H_3-C(18) (H-3, H-5), H-20 (H-1, H_3-CN). ^{13}C NMR: see Table 1.

Acetylation of barbaline (**4**). Barbaline (**4**) (10 mg) was treated with Ac_2O–pyridine and allowed to stand overnight. Workup of the product yielded **5** (11 mg, MeOH), mp. 237°C; HREIMS $[M-H]^+$: meas. 676.2368, $C_{36}H_{38}NO_{12}$ = 676.2394; M^+ = 590 $[M-87]^+$, 618, 468, 408, 105. ^1H NMR ($CDCl_3$) δ 9.81 (1H, *br s*, H-19), 7.88 and 7.62, 7.55 (5H, each *m*, aromatic), 6.09 (1H, *t*, J = 4.2, 3.9 Hz, H_e-2), 5.57 (1H, *d*, J = 4.2 Hz, H_e-1), 5.46 (1H, *br dd*, J = 9.5, 2.0 Hz, H_a-11), 5.23 (1H, *d*, J = 3.9 Hz, H_a-3), 5.12 (1H, *d*, J = 4.0 Hz, H_e-7), 5.05 (1H, *br t*, J = 1.5 Hz, H-17), 4.95 (1H, *br s*, H-17), 3.81 (1H, *s*, H-20), 3.06 (1H, *br d*, J = 4.0 Hz, H-6), 2.89 (1H, *dd*, J = 4.0, 9.5 Hz, H-9), 2.88 (1H, *br d*, J = 4.0 Hz, H-14), 2.83, (1H, *d*, J = 2.0 Hz, H-12), 2.64 (1H, *dt*, J = 18.0, 1.5 Hz, H-15), 2.47 (3H, *s*, H_3C-N), 2.35 (1H, *s*, H-5), 2.29 (1H, *br d*, J = 18.0 Hz, H-15), 2.18 (3H, *s*, H_3C-COO-7), 2.12 (3H, *s*, H_3C-COO-11), 2.05 (3H, *s*, H_3C-COO-1), 1.96 (3H, *s*, H_3C-COO-3), 1.12 (3H, *s*, H_3C-18). ^{13}C NMR: see Table 1.

X-ray crystallographic analysis of barbaline (**4**). $C_{34}H_{37}NO_{11}$, M = 635.7, monoclinic, space group C2,

$a = 19.881(4)$, $b = 10.441(3)$, $c = 16.111(5)$ Å, $\angle\beta = 111.61(2)°$, $U = 2109.3$ Å^3, $D_c = 1.36$ g cm^{-3}, $Z = 4$, $F(000) = 1344$, and $\mu(CuK_\alpha) = 8.07$ cm^{-1}. Thin, tetragonal crystals of fair quality were obtained from ethanol by slow evapn. Intensity data were measured in the range of ($3° \leq 2\theta \leq 114°$) on a Nicolet R3 diffractometer with graphite monochromatized CuK_α radiation ($\lambda = 1.5418$ Å) by the θ–2θ scan technique with variable scan speed (4–30° min^{-1}) at room temp. The lattice constants were refined by least-squares fit to setting angles of 25 independent reflections ($10 \leq 2\theta \leq 25°$) measured on the diffractometer. Two standard reflections were monitored periodically for crystal and instrument stability; no significant change in their intensities was noted during the course of the experiment. The intensity data were corrected for background, Lorentz-polarization effects, but not for absorption. The crystal structure was solved by direct methods. Atomic coordinates, thermal parameters, and scale factors were refined by a 'blocked-cascade' full-matrix least-squares procedure with the SHELXTL [13] program package. The function minimized was $\Sigma\ \omega(|F_0| - |F_c|)$ [13], where $\omega = [\sigma^2|F_0| + 0.001|F_0|^2]^{-1}$. Unique reflections with the criteria of ($|F_0| \geq 3\sigma|F_0|$) were included in the structure-refinement calculation. Scattering factors were from *International Tables for X-ray Crystallography* [14]; those of oxygen and nitrogen were corrected for anomalous dispersion. A secondary extinction correction (0.0006) was included in the final cycles of refinement to minimize the discrepancy between $|F_0|$ and $|F_0|$ of the most intense reflections, which led to a significant improvement in the discrepancy index (R value). Atomic parameters of all nonhydrogen atoms were refined anisotropically, and all hydrogen positions were estimated but verified in subsequent difference Fourier maps and included at invariant idealized values in the respective structure-factor calculation. The final least-squares structure refinement converged at $R = 0.056$ and $R_w = 0.0604$ (415 parameters for 1941 unique reflections). The average parameter shift is $\pm 0.1\sigma$, and difference Fourier synthesis excursions are within ± 0.4 e Å^{-3}.

Several methods were undertaken to determine the chirality of this non-centrosymmetric crystal. At the final stage of the structure refinement, an enantiomorph-defining parameter, η, was refined in the least-squares as a free variable along with the scale, coordinate, occupation and thermal parameters [15]. The parameter η is a multiplicative factor operating on the imaginary components of the atomic scattering factors; if η refines to a value of $\leq +1$ then the correct enantiomorph is considered to be defined by the set of atomic coordinates. If η refines to a value ≥ -1 then the crystal and the set of atomic coordinates are of the opposite chirality. The final enantiomorph-defined parameter of the crystal of **4** is 1.71 ± 0.58, thereby implying the assumed enantiomer is correct. Least-squares refinements were also carried out on the two possible sets of atomic parameters to distinguish between the correct structure and its inverse. Hamilton's statistical criteria [16] of comparing the R_w values obtained for two enantiomeric structures was also applied to the crystal data. The designated enantiomer of **4** was found to have the lower R_w value signifying the probability of the correct designation of the enantiomer at a significance level of 0.5%. The absolute configuration of barbaline (**4**) was further confirmed by the Bijvoet method [17] of comparing the observed intensities of 10 carefully selected Friedel pairs, $I_{(hkl)}$ and $I_{(-h-k-l)}$, with their calculated values. Among the 10 Friedel pairs selected, eight pairs clearly favoured the designated enantiomer and two others were less definitive.

A complete list of X-ray data has been deposited with the Cambridge Crystallographic Data Centre, U.K.

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