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REDUCTION PRODUCTS OF THE ALKALOID NORFLUOROCURARINE

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The alkaloid norfluorocurarine was reduced under various conditions to produce the novel bisindoline derivative 2,16-dihydro-19-oxo-C-dihydrotoxiferine and the previously known natural derivatives deoxydihydro-, deoxytetrahydro-, and tetrahydronorfluorocurarine. The structures of the synthesized compounds were identified using IR and UV spectroscopy; of newly produced 2,16-dihydro-19-oxo-C-dihydrotoxiferine and deoxydihydronorfluorocurarine, also by x-ray structure analysis. Determination of the absolute configuration of N*(α)-methylfluorocurarine chloride dihydrate by x-ray methods enabled the configuration of the 3*S*,7*R*,15*S *asymmetric centers in norfluorocurarine and 2*S*,3*S*,7*R*,15*S *in deoxydihydronorfluorocurarine to be determined. Also, the absolute configuration of 2,16-dihydro-19-oxo-C-dihydrotoxiferine was determined as 2*S*,3*S*,7*R*,15*R*,16*R *in one part of the bisindoline and 3′*S*,7′*R*,15′*S *in the other.*

Keywords: indoline alkaloids, norfluorocurarine, 2,16-dihydro-19-oxo-C-dihydrotoxiferine, absolute configuration, x-ray structure analysis.

The indoline alkaloid norfluorocurarine (**1**), $C_{19}H_{20}N_2O$, was first isolated from *Vinca erecta* roots as a new base named vincanine [1, 2]. Later, after we established its structure [3], it became apparent that vincanine was identical to the alkaloid norfluorocurarine, which was isolated earlier from the plants *Diplorzhynos condilacarpon* [4] and *Strychnos toxifera* [5, 6]. Its bimolecular analogs with the common name toxiferines and the racemic mixture (±)-norfluorocurarine with the name vinervidine are well known [7].

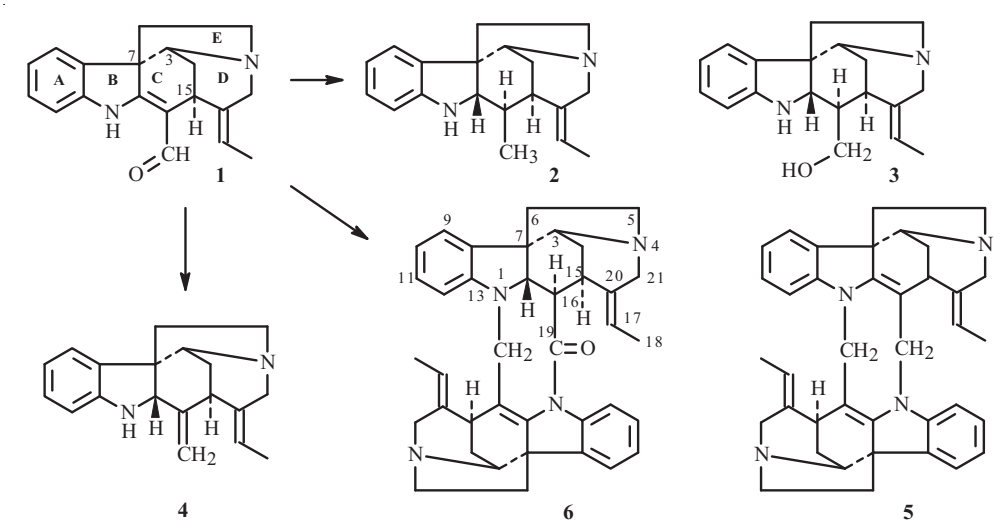

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TABLE 1. Intermolecular H-bonds in **1′** and **4**

Atom	D-H (Å)	H…A (Å)	D…A (Å)	D–H < A (°)	Symmetry transf.
		Crystal of **1′**			
Ow1–H2 … O	0.89	2.07	2.925	163	–
Ow2–H1 … Cl	0.81	2.52	3.320	174	–
Ow2–H2 … Ow1	0.84	2.05	2.883	173	1 + x, y + 1, z
Ow1–H1 … Cl	0.84	2.37	3.189	168	x, y + 1, z
		Crystal of **4**			
N1-H… N4	0.86	2.28	3.041	148	–x, y + 1/2, –z

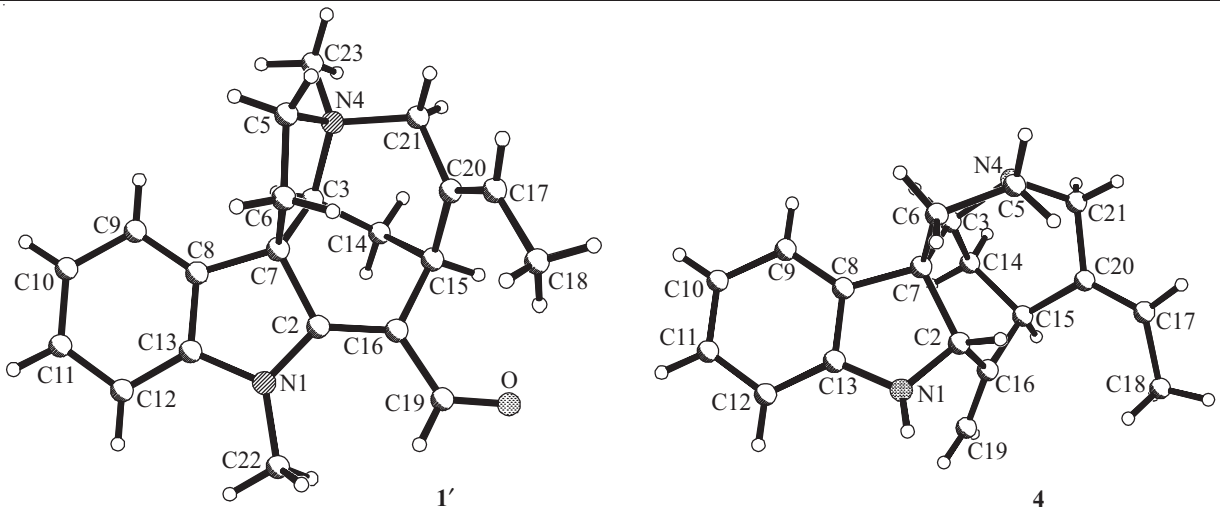

Fig. 1. Molecular structure and atomic numbering of the molecular cation in **1′** and product **4**.

Indoline alkaloids of this series exhibit a broad spectrum of biological activity. In particular, alkaloids such as reserpine, ajmaline, vincristine, vinblastine, vincamine, etc. are widely used in medicine as valuable drugs [8].

In order to find new biologically active compounds in the norfluorocurarine series, we reduced it under various conditions. Norfluorocurarine reduced by sodium in anhydrous alcohol formed deoxytetrahydronorfluorocurarine $C_{19}H_{24}N_2$ (**2**) and tetrahydronorfluorocurarine $C_{19}H_{24}N_2O$ (**3**). The latter turned out to be identical to the Wieland–Gumlich 18-deoxyglycol that was prepared from strychnine [3, 5]. Norfluorocurarine reduced by Zn in H_2SO_4, $NaBH_4$, and $LiAlH_4$ formed deoxydihydronorfluorocurarine (**4**) [3]. An analogous compound was prepared from the alkaloid akuammicine and was called anhydro-2,16-dihydroakuammicinol [9].

Reduction of **1** by Zn in H_2SO_4 on a sand bath produced **4** and a novel bimolecular base of formula $C_{38}H_{40}N_4O$. The literature contains many examples of bisindoline alkaloids with different bonding of the monomers [10–13]. A similar but symmetric bimolecular base of formula $C_{38}H_{38}N_4$ was isolated earlier from total alkaloids of gourd curare and was called nordihydrotoxiferine (**5**) [14]. The structure of the new bisindoline derivative was established by analogy as 2,16-dihydro-19-oxonordihydrotoxiferine (**6**) based on an x-ray structure analysis (XSA). The aforementioned synthesized compounds were also identified using IR and UV spectral methods.

The stereochemistry of these alkaloids was previously studied by physicochemical methods. The relative configurations of the asymmetric centers were determined but the absolute (*R*,*S*)-configuration was not established. In particular, **1** has C3, C7, and C15 asymmetric centers. Another one at C2 is added in **4**; at C16, in **6**. For this reason, the salt *N*(α)-methylfluorocurarine chloride (**1′**) was prepared and its absolute structure was determined by XSA in order to resolve these stereochemistry issues. Herein we report results from the structural studies.

Figures 1 and 2 show the molecular structures of the cation in **1′**, the reduction product of norfluorocurarine **4**, and the bimolecular derivative **6** from XSAs. The absolute configurations of asymmetric centers C3, C7, and C15 in **1** corresponded to those in Fig. 1 for the molecular cation in **1′**. These centers had the 3*S*, 7*R*, and 15*S* configurations according to the structure and the Flack parameter of 0.03(2). Atom N4 in the molecular cation of **1′** was methylated, as a result of which it became an asymmetric center with the 4*S* configuration. The absolute structure of **4** was not established by XSA. However, it was the reduction product of **1**. Therefore, the asymmetric centers in **4** had the 2*S*, 3*S*, 7*R*, and 15*S* configurations (Fig. 1).

TABLE 2. Principal Crystallographic Data and X-ray Structure Characteristics for **1′**, **4**, and **6**

Structure	**1′**	**4**	**6**
Molecular formula	$C_{21}H_{25}ON_2 \cdot Cl \cdot 2H_2O$	$C_{19}H_{22}N_2$	$C_{38}H_{40}ON_4$
MW, g/mol	392.91	278.39	568.74
System	Orthorhombic	Orthorhombic	Monoclinic
Space group	$P\ 2_12_12_1$	$P\ 2_12_12_1$	$P\ 2_1$
Z	4	4	2
a, Å	6.6667 (2)	7.7115 (1)	11.2092 (3)
b, Å	11.5166 (3)	13.4090 (2)	10.8878 (2)
c, Å	26.0495 (7)	14.8139 (3)	12.0859 (4)
α	90.00	90.00	90.00
β	90.00	90.00	92.834 (3)
γ	90.00	90.00	90.00
V, Å^3	2000.02 (10)	1531.81 (4)	1473.21 (7)
ρ, g/cm^3	1.305	1.207	1.282
Crystal size, mm	0.30 × 0.25 × 0.15	0.4 × 0.4 × 0.3	0.5 × 0.4 × 0.2
2θ scan range	3.39 ≤ θ ≤ 75.74°	4.45 ≤ θ ≤ 75.40°	3.66 ≤ θ ≤ 70.92°
μ_{exp}, cm^{-1}	1.881	0.540	0.603
Number of reflections	3587	3068	4292
Number of reflections with $I > 2\ \sigma$ (I)	2809	2765	3617
R_1 ($I > 2\ \sigma$ (I) and total)	0.0440 (0.0612)	0.0465 (0.0510)	0.0403 (0.0488)
WR_2	0.1039 (0.1125)	0.1346 (0.1382)	0.0990 (0.1029)
GOOF	0.975	1.054	0.965
Electron-density difference peaks, e/Å^{-3}	0.18 and –0.18	0.25 and –0.26	0.16 and –0.17
CCDC	771515	771516	771517

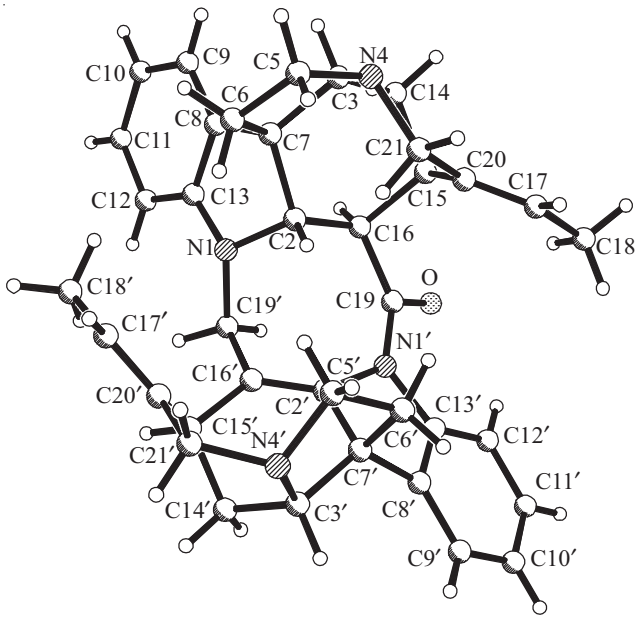

Fig. 2. Molecular structure and atomic numbering of **6**.

An analogous approach was used to determine the absolute configuration of the bisindoline derivative 2,16-dihydro-19-oxo-C-dihydrotoxiferine (**6**). Compound **6** was formed by addition of norfluorocurarine and deoxydihydronorfluorocurarine with known absolute structures at nodal atoms N1 and C19. For this reason and judging from the molecular structure (Fig. 2), it can be confirmed that the asymmetric centers in 2,16-dihydro-19-oxo-C-dihydrotoxiferine had the 2*S*, 3*S*, 7*R*, 15*R*, 16*R* configurations in one indoline part (like in product **2**) and 3′*S*, 7′*R*, 15′*S*, in the other.

The molecular cation of **1′** consisted of five condensed rings where rings C/E and E/D were *cis* fused. The absolute configuration of the asymmetric centers and the ring fusions agreed with those in the related alkaloid strychnine [15]. The

indoline core (rings A and B) with the N1-methyl was planar within ±0.033 Å due to the presence in the heterocycle of sp^2-hybridized atom C2. Ring C with endocyclic double bond C2=C16 [1.384(4) Å] adopted a distorted boat conformation (twist-boat) with C2 symmetry (passing through the middle of bond pairs C2–C7 and C14–C15). Ring D had a slightly distorted boat conformation. Five-membered ring E adopted the envelope conformation with atom C5 deviating by 0.549 Å from the plane of the remaining atoms (±0.018 Å).

The C2–C16 bond in **4** was reduced [bond length 1.526(3) Å]. The new B/C ring fusion was *cis*. Rings in other instances were fused the same as in **1′**. Compound **4** exhibited slight conformational differences from **1′**. The indoline core was not completely planar because ring B adopted the envelope conformation with atom C2 deviating by 0.439 Å from the plane of the other atoms in the indoline core. Ring C had a slightly distorted chair conformation. Ring D had an ideal boat shape. Five-membered ring E adopted the envelope conformation but, in contrast with **1′**, atom C6 deviated by 0.628 Å from the plane of the other four atoms (±0.046 Å).

The indoline cores in **6** were also not completely planar. Five-membered rings B and B′ adopted the envelope conformation with atoms C2 and C2′ deviating from the plane of the other four atoms by 0.524 and 0.488 Å, respectively. Although the exocyclic double bond C2′=C16′ in one indoline part of **6** was retained [1.325(3) Å], it was reduced in the other [1.529(3) Å]. Therefore, the ring fusions in the monomers were different (B/C, *trans*; C/E and E/D, *cis* in one indoline part; C/E and E/D, *cis* in the other). Rings C and C′, in contrast with **1′**, adopted a slightly distorted chair and ideal chair conformations with atom C14′ deviating from the plane of the other five atoms (±0.020 Å) by 0.737 Å. Rings D and D′ also had different conformations, a chair and slightly distorted boat, respectively. Five-membered heterocycles E and E′ had envelope conformations with atoms C3 and C6′, respectively, deviating from the planes.

Molecular Packing. Crystals of *N*(α)-methylfluorocurarine chloride turned out to be the dihydrate. Two waters of crystallization in the unit cell of **1′** formed an infinite chain of intermolecular H-bonds involving the Cl anion and the carbonyl O atom of the alkaloid (cation) along the crystallographic *a* axis. Table 1 lists the parameters of these H-bonds.

The crystal of **4** contained an N–H...N intermolecular H-bond. Table 1 lists the parameters of this H-bond. An infinite screw chain along the crystallographic *b* axis was formed. In contrast with **1′** and **4**, the molecules in the crystal of **6** were situated at van-der-Waals distances.

EXPERIMENTAL

IR spectra were recorded in KBr pellets on a Model 2000 Fourier IR spectrometer (Perkin–Elmer). UV spectra were measured on a Lambda-16 spectrophotometer (Perkin–Elmer).

2,16-Dihydro-19-oxo-C-dihydrotoxiferine (6). Norfluorocurarine (**1**, 1 g) in H_2SO_4 solution (20 mL, 20%) was heated on a sand bath with granular Zn for 1.5 h. The solution gradually lightened and was cooled, made basic with NaOH solution (10%), and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The solid was treated with acetone. The crystalline precipitate was recrystallized from Me_2CO:MeOH (3:1), mp 297–300°C (dec.), $C_{38}H_{40}N_4O$, R_f 0.20 ($CHCl_3$:MeOH, 9:1). IR spectrum (ν, cm^{-1}): 1602 (C=N), 1676 (C=O). UV spectrum (λ_{max}, nm, log ε): 258 (3.98), 3.06 (3.24).

Deoxydihydronorfluorocurarine (4). The mother liquor (900 mg) remaining after isolation of **6** was dissolved in benzene and chromatographed over a column of Al_2O_3 with elution by benzene. The first fractions afforded after distillation of benzene crystals, mp 193–194°C, $C_{19}H_{22}N_2$, $[\alpha]_D^{18}$ –38.8° (*c* 0.88, MeOH). IR spectrum (ν, cm^{-1}): 3175 (N–H), 1650–1614 (C=CH–CH_3). UV spectrum (λ_{max}, nm, log ε): 250 (3.84), 300 (3.50).

Tetrahydronorfluorocurarine (3). Compound **1** (1 g) was dissolved in anhydrous alcohol (60 mL), refluxed, treated in portions with metallic Na (5 g) over 2 h, diluted with water, and extracted with ether. The extract was washed with water, dried over anhydrous Na_2SO_4, evaporated to dryness, and treated with benzene to isolate a crystalline compound (350 mg), mp 174–175°C, $C_{19}H_{24}N_2O$, $[\alpha]_D^{20}$ –28.5° (*c* 1.51, MeOH). IR spectrum (ν, cm^{-1}): 3665 (OH), 3355, 3226 (N–H), 1660–1610 (C=CH–CH_3). UV spectrum (λ_{max}, nm, log ε): 245 (3.80), 298 (3.40).

Deoxytetrahydronorfluorocurarine (2). The mother liquor after isolation of **3** was evaporated to dryness. The solid was recrystallized from Me_2CO to afford **2** (150 mg), mp 185–186°C, $C_{19}H_{24}N_2$, $[\alpha]_D^{20}$ –61.1° (*c* 2.43, MeOH). IR spectrum (ν, cm^{-1}): 3405 (N–H), 1656–1616 (C=CH–CH_3). UV spectrum (λ_{max}, nm, log ε): 250 (3.84), 300 (3.50).

***N*(α)-Methylfluorocurarine Chloride Dihydrate (1′).** Compound **1** (1 g) was dissolved in water (30 mL) and MeOH (10 mL), stirred vigorously, treated from two burettes with NaOH solution (30 mL, 2 N) and dimethylsulfate (10 mL) over 3 h, and treated with an excess of sodium picrate solution. The resulting crystals (930 mg) were recrystallized from aqueous Me_2CO, mp 209–210°C, $C_{21}H_{25}N_2O{\cdot}C_6H_2O_7N_3$.

N(α)-Methylfluorocurarine chloride picrate (500 mg) was dissolved in aqueous Me_2CO and passed over a column packed with Amberlite IRA-400 resin (Cl^--form) with elution by aqueons Me_2CO. The solvent was vacuum distilled. The solid (160 mg) was recrystallized from MeOH to afford **1′**, mp 268–270°C, $C_{21}H_{25}N_2O{\cdot}Cl{\cdot}2H_2O$. UV spectrum ($\lambda_{max}$, nm, log ε): 242 (4.10), 301 (3.76), 356 (4.35).

X-ray Structure Analysis (XSA). Single crystals for XSA were grown by slow evaporation from the appropriate solvents at room temperature. The unit-cell constants for **1′**, **4**, and **6** were determined and refined on a CCD Xcalibur Ruby diffractometer (Oxford Diffraction) using Cu K_α-radiation (300 K, graphite monochromator). A three-dimensional data set of reflections was obtained on the same diffractometer. Absorption corrections were applied using the semi-empirical method of the SADABS program [16]. Table 2 presents the principal x-ray structure parameters and refinement parameters for the structures of **1′**, **4**, and **6**.

The structures were solved by direct methods using the SHELXS-97 program set and refined using the SHELXL-97 program. All non-hydrogen atoms were refined by anisotropic full-matrix least-squares techniques (over F^2). The positions of H atoms were found geometrically and refined with fixed isotropic thermal parameters $U_{iso} = nU_{eq}$, where $n = 1.5$ for methyls and 1.2 for others and U_{eq} are the equivalent isotropic thermal parameters of the corresponding C atoms. The H atoms in the waters of crystallization in the crystal of **1′** were found in a difference electron-density synthesis and refined isotropically.

The XSA data were deposited as CIF files in the Cambridge Crystallographic Data Centre (CCDC).

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