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SYNTHESES BASED ON NORFLUOROCURARINE.
4. REACTIONS WITH HYDROXYLAMINE

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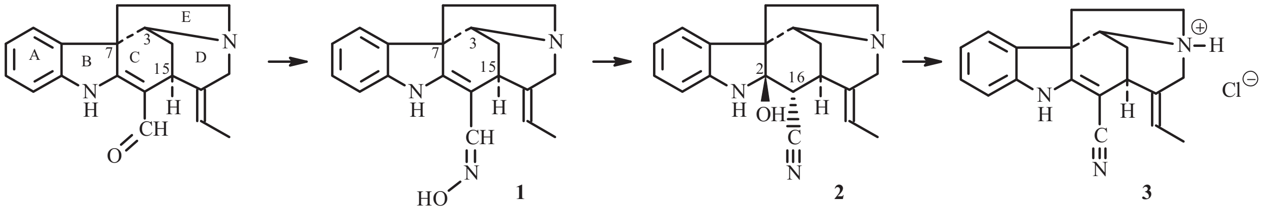
*Transformation products of the alkaloid (–)-norfluorocurarine with hydroxylamine were studied. The oxime of norfluorocurarine was obtained and converted by treatment with EtOH into 2-hydroxy-16-cyano-2,16-dihydronorfluorocurarine. The structures of 2-hydroxy-16-cyano-2,16-dihydronorfluorocurarine and 16-cyanonorfluorocurarine hydrochloride were established by XSA and NMR spectroscopy. The absolute structure of 2-hydroxy-16-cyano-2,16-dihydronorfluorocurarine was established and enabled the configurations of the asymmetric centers to be determined as 2*R*, 3*S*, 7*R*, 15*S*, and 16*R*.*

Keywords: indoline alkaloids, norfluorocurarine, absolute configuration, XSA.

In continuation of research on the production of new biologically active derivatives of the indoline alkaloid (–)-norfluorocurarine [1, 2], we studied its reaction with hydroxylamine. Norfluorocurarine has a reactive aldehyde conjugated with a double bond. A mixture of three compounds, the principal components of which were starting (–)-norfluorocurarine and its oxime (**1**), was formed from its reaction with hydroxylamine according to TLC and NMR spectroscopy. A compound whose structure could not be solved occurred in small quantities. The UV spectrum of the mixture had three maxima that were characteristic of the HN–C=C–C=N–OH chromophore. Treatment of the mixture with alcohol or storage of it formed in it mainly 2-hydroxy-16-cyano-2,16-dihydronorfluorocurarine (**2**). The formation of indoline compound **2** could be explained by an intramolecular rearrangement with hydration of the C2=C16 double bond. Acidification of **2** by HCl solution in alcohol produced 16-cyanonorfluorocurarine hydrochloride (**3**). The sequence of transformations of (–)-norfluorocurarine is shown in Scheme 1.



Scheme 1

The IR spectrum of the products contained absorption bands characteristic of indoline alkaloids. The UV spectrum of **2** had two maxima that were typical of compounds with the indoline structure. The spectrum of **3** had three maxima characteristic of compounds with the α-methylindoline structure (see Experimental). X-ray structure analysis (XSA) was used to determine positively the structures and to resolve stereochemistry issues of **2** and **3**.

Compound **1** could not be isolated pure and crystals could not be grown. Therefore, PMR spectroscopy was also used to prove the presence in the initial reaction mixture of norfluorocurarine, its oxime **1**, and the unidentified compound. Compounds **2** and **3** were analyzed pure in $CDCl_3$. Table 1 presents their chemical shifts and the splitting pattern of the resonances according to SSCC for starting norfluorocurarine. The splitting pattern of the resonances for **2** and **3** had similar values. Therefore, they are not given in Table 1. A comparison of PMR spectra of the initial reaction mixture and spectra of **2** and **3** confirmed the TLC data about the presence in the solution of three compounds and identified several resonances belonging to **1**.

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TABLE 1. PMR Spectra of Norfluorocurarine and **1**, **2**, and **3** in $CDCl_3$ with HMDSO Internal Standard (0 ppm)

Atom	Norfluorocurarine	**1**	**2**	**3**	SSCC, Hz
3	4.017		3.261	3.188	3.6 (14α), 2.2 (14β), 1.9 (15)
5α	3.230		3.092	3.034	12.5 (5β), 12.5 (6β), 5.5 (6α)
5β	2.994		2.843	2.817	12.5 (5α), 6.6 (6β), 1.3 (6α)
6α	1.746		2.128	2.080	12.4 (6β), 5.5 (5α), 1.3 (5β)
6β	2.307		2.581	2.627	12.4 (6α), 12.5 (5α), 5.5 (5β)
9	7.212	6.210	7.051	7.020	7.5 (10), 1.4 (11), 0.6 (12)
10	6.892	6.630	6.809	6.763	7.5 (9), 7.6 (11), 0.8 (12)
11	7.117	6.615	7.071	7.030	7.7 (12), 7.6 (10), 1.4 (9)
12	6.844	6.640	6.642	6.634	7.7 (11), 0.8 (10), 0.6 (9)
14α	1.203		1.597	1.561	13.7 (14β), 3.6 (3), 2.2 (15)
14β	2.497		2.106	2.140	13.7 (14α), 4.1 (15), 2.2 (3)
15	3.628		3.215	3.190	m
16	–		2.759	–	–
17	5.319		5.346	5.364	3 × 6.9 (18), 2.3 (15), 2.3 (21β)
18	1.524	1.495	1.661	1.659	6.9 (17), 2.5 (21β)
19	10.291	9.196	–	–	s
21α	2.857		3.012	3.019	15.8 (21β)
21β	3.921		3.441	3.391	15.8 (21α), 3 × 2.5 (18), 2.3 (17), 1.7 (15)

These were primarily a singlet for vinyl proton H-19 (9.196 ppm) and characteristic resonances of aromatic protons H-9–H-12. The resonance of the H-18 methyl of **1** at 1.495 ppm could also be identified. The remaining resonances of **1** could not be identified individually because of the slight differences with respect to **2** and **3** and the strong overlap. However, the presence in the initial solution of a pure compound that should be **1** can be considered fully justified.

The C2=C16 double bond was saturated upon isomerization of **1** (conversion to **2**). [According to an XSA, the C2–C16 bond length in **2** is 1.554(4) Å.] This could theoretically lead to the formation of four diastereomers at asymmetric C2 and C16. The absolute configuration of the asymmetric centers in the (–)-norfluorocurarine heterocyclic framework was examined earlier and is considered known [1]. These reactions did not affect centers 3*S*, 7*R*, and 15*S* in the norfluorocurarine skeleton. Therefore, the XSA enabled the configuration of the other asymmetric center in **2** to be determined relative to the known ones.

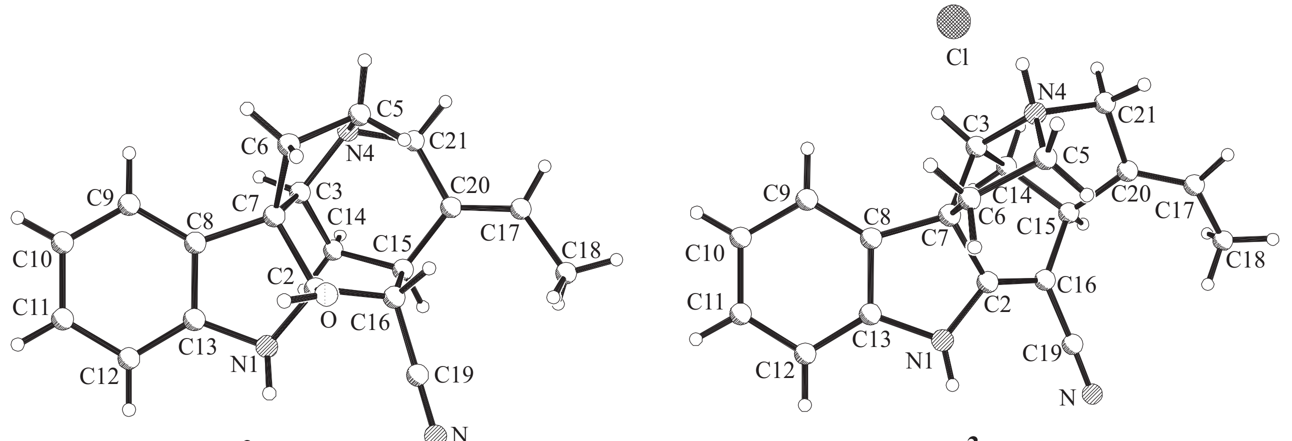
Figure 1 shows the steric structures of **2** and **3** in the same projection for the plane of the indole ring. The asymmetric centers of **2** according to the given structure are defined as 2*R*, 3*S*, 7*R*, 15*S*, and 16*R*. According to this, the name of the alkaloid should be refined as 2β-hydroxy-2α,16β(*H*)-dihydro-16-cyanonorfluorocurarine. The absolute configuration of the molecule in the structure of salt **3** was confirmed experimentally by the Flack parameters [0.09(8)]. Therefore, the chiral atoms, as expected, retained the 3*S*, 7*R*, 15*S* configuration whereas the protonated N4 acquired the 4*S* configuration.

Five-membered ring B in the indoline cores of **2** and **3** adopted a flattened 2β-, 2α-envelope conformation with C2 deviating from the plane of the other core atoms (determined with accuracy ±0.009 and ±0.031 Å) by –0.480 and 0.415 Å, respectively. Ring C in **2** was a distorted boat. Atoms C2 and C14 deviated from the plane of the other four (±0.034 Å) by 0.420 and 0.739 Å, respectively. However, this ring in **3** had the half-chair conformation because of the presence of the C2=C16 double bond [1.340(9) Å]. Here the deviation of C14 from the plane of the other five atoms (±0.033 Å) was 0.766 Å. Ring D in both molecules adopted the same slightly distorted boat conformation. It was flattened on the side of C21. Ring E was a 6β-envelope with C6 deviating by –0.576 and –0.583 Å from the plane of the other atoms (±0.015 and ±0.033 Å), respectively. The fusion of the rings in **2** was B/C- C/E-, and E/D-*cis*.

Molecular Packing. Intermolecular H-bonds of the OH…N type between hydroxyl H atoms on C2 of the starting molecule and tertiary N4 transformed by $P2_1$ symmetry ($x, -1 + y, z$) were observed in the crystal structure of **2**. The parameters of this H-bond were O…N4, 2.764(4); H…N4, 1.98 Å; O–H…N4 angle, 163°. As a result, an infinite chain along the *b* screw axis formed in the crystal of **2**. Intermolecular H-bonds in the crystal structure of **3** were formed through the Cl^- anion, i.e., H-bonds of the N–H…Cl…H–N type were formed with the N1H and N4H groups where the second NH group was obtained by transformation of the molecule by the 2_1 screw axis ($1 - x, -1/2 + y, 1/2 + z$). The parameters of these H-bonds were N1H…Cl, 3.180(8), 2.37 Å, and 157° and N4H…Cl, 2.990(8), 2.10 Å, and 157°, respectively. As a result, infinite chains along the *b* screw axis also formed in the crystal of **3**.

TABLE 2. Principal Crystallographic Parameters and Characteristics of X-ray Structure Experiment for **2** and **3**

Structure	**2**	**3**
Molecular formula	$C_{19}H_{21}N_3O$	$C_{19}H_{20}N_3Cl$
MW, g/mol	307.39	325.83
System	Monoclinic	Orthorhombic
Space group	$P\,2_1$	$P\,2_12_12_1$
Z	2	4
a, Å	7.4053 (7)	7.620 (1)
b, Å	12.579 (1)	14.752 (3)
c, Å	8.5190 (9)	14.760 (2)
α	90.00	90.00
β	93.049 (8)	90.00
γ	90.00	90.00
V, Å^3	792.45 (13)	1659.1 (5)
ρ, g/cm^3	1.288	1.304
Crystal size, mm	$0.6 \times 0.2 \times 0.1$	$0.4 \times 0.3 \times 0.1$
Scan range	$5.98 \le \theta \le 70.79°$	$4.24 \le \theta \le 67.11°$
μ_{exp}, cm^{-1}	0.642	2.044
Number of reflections	1939	2034
Number of reflections with $I > 2\sigma(I)$	1680	1199
R_1 ($I > 2\sigma(I)$ and total)	0.0422 (0.0503)	0.0593 (0.1162)
WR_2	0.1156 (0.1213)	0.1250 (0.1487)
GOOF	0.964	0.934
Difference electron-density peaks (e Å^{-3})	0.18 and –0.15	0.34 and –0.21
CCDC	818499	818500



2 **3**

Fig. 1. Molecular structure and atomic numbering of **2** and **3**.

EXPERIMENTAL

IR spectra were taken in KBr pellets on a Model 2000 Fourier IR-spectrometer (Perkin–Elmer). UV spectra were measured on a Lambda-16 spectrophotometer (Perkin–Elmer). NMR spectra were recorded in $CDCl_3$ with HMDSO internal standard (0 ppm) on a Unity 400+ spectrometer (Varian) at operating frequency 400 MHz.

Norfluorocurarine Oxime (1). Pure anhydrous norfluorocurarine hydrochloride (600 mg) was dissolved in H_2O (30 mL), mixed with a solution of hydroxylamine sulfate (250 mg) in H_2O (30 mL), heated on a boiling water bath for 20 min, cooled, made basic with NaOH solution (5%), and extracted with Et_2O. The Et_2O extract was dried over anhydrous Na_2SO_4 and evaporated to dryness to afford a mixture (620 mg) consisting (according to TLC) of norfluorocurarine, its oxime (**1**), and an unidentified compound in minor amounts. The reaction products were a mixture of three compounds with R_f values 0.49, 0.36, and 0.23 ($CHCl_3$:MeOH, 1:1). IR and UV spectra were recorded.

IR spectrum (ν, cm^{-1}): 3456 (OH), 3299 (NH), 2743 (CH=N), 1642 (C=N), 1610, 1588 (C=C), 926 (N–OH). UV spectrum (λ_{max}, nm): 203, 236, 294, 364.

2-Hydroxy-16-cyano-2,16-dihydronorfluorocurarine (2), consisting of norfluorocurarine and its oxime **1** and the unidentified compound, was dissolved in a mixture of EtOH and acetone. Crystals (120 mg) formed on standing. Recrystallization from EtOH afforded colorless prisms of 2-hydroxy-16-cyano-2,16-dihdyronorfluorocurarine, mp 169–170°C, $C_{19}H_{21}ON_3$.

IR spectrum (ν, cm^{-1}): 3376 (OH), 3327 (NH), 3047 (H–C=C), 2234 (C≡N), 1606 (C=C), 1463, 1434 (C=C–C_{ar}), 1279, 1130 (C–N). UV spectrum (λ_{max}, nm): 238, 292.

16-Cyanonorfluorocurarine Hydrochloride (3). 2-Hydroxy-16-cyano-2,16-dihydronorfluorocurarine (2.4 g) in EtOH (40 mL) was heated gently and treated with HCl in alcohol (5.5 mL). Cooling produced crystals of **3**, $C_{19}H_{20}N_3Cl$, 1.95 g after recrystallization from H_2O, mp 214–215°C, R_f 0.6 ($CHCl_3$:MeOH, 1:1), $[\alpha]_D^{20}$ –434.7° (*c* 2.98, MeOH).

IR spectrum (ν, cm^{-1}): 3146 (NH), 3075 (H–C=C), 2453 (N^+–H), 2193 (C≡N), 1624, 1606 (C=C), 1479, 1461 (C=C–C_{ar}), 1207, 1049 (C–N). UV spectrum (λ_{max}, nm): 224, 290, 319.

XSA. Single crystals for XSA were grown by slow evaporation from the appropriate solvents at room temperature. Unit cell constants of crystals of **2** and **3** were determined and refined on a CCD Xcalibur Ruby diffractometer (Oxford Diffraction) using Cu Kα-radiation (300 K, graphite monochromator). A three-dimensional data set of reflections was obtained on the same diffractometer. Absorption corrections were applied by a semi-empirical method using the SADABS program [3]. Table 2 presents the principal parameters of the XSA experiments and refinement calculations for the structures of **2** and **3**.

The structures were solved by direct methods using the SHELXS-97 program set. Structures were refined using the SHELXL-97 program and displayed graphically using the SHELXTL program [4]. All nonhydrogen atoms were refined by anisotropic full-matrix least-squares methods (over F^2). Positions of H atoms were found geometrically and refined with fixed isotropic thermal parameters $U\text{-}_{iso} = nU_{eq}$, where n = 1.5 for methyls and 1.2 for others, and U_{eq} was the equivalent isotropic thermal parameter of the corresponding C atoms.

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

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