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GENESERINE AND GENESEROLINE REVISITED: ACID-BASE CATALYZED EQUILIBRIA OF HEXAHYDROPYRROLO-[2,3-*b*]-INDOLE *N*-OXIDES WITH HEXAHYDRO-1,2-OXAZINO-[5,6-*b*]-INDOLES

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ABSTRACT.—It is shown that geneserine [**2**] and geneseroline [**4**] convert with acid into salts of physostigmine *N*-oxide [**3**] and geneseroline *N*-oxide [**5**], respectively. Structure assignments were made on the basis of chemical conversions, ^{1}H-nmr analysis of **2**–**5**, and an X-ray analysis of **5** as its hydrochloride.

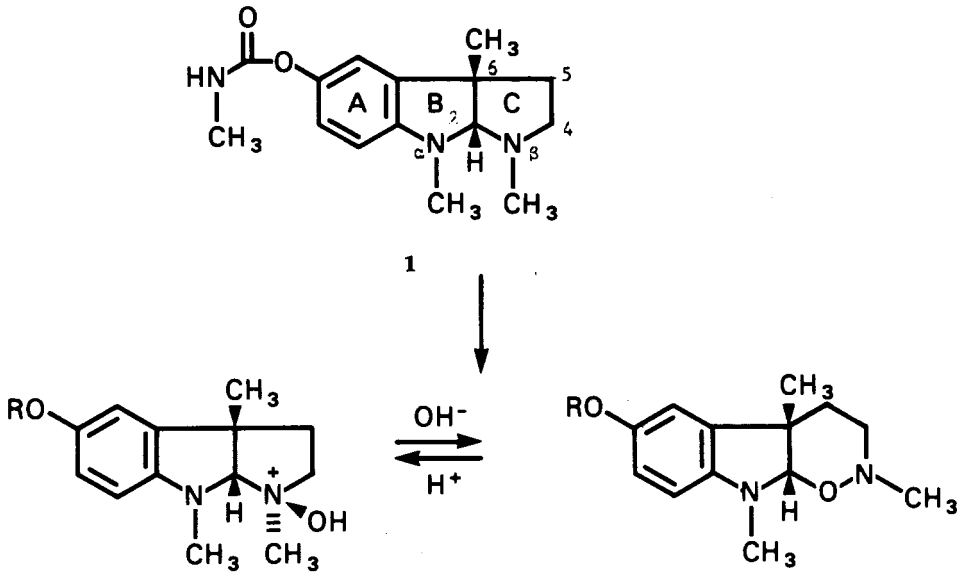
The alkaloid geneserine [**2**] was first isolated by Polonovski from the basic extracts of *Physostigma venenosum* seeds (1) and later obtained from physostigmine [**1**] by oxidation with H_2O_2 (2). Structure **2** for geneserine with a *cis*-fused tetrahydro-1,2-oxazine ring [versus the *N*-oxide structure **3** proposed by Polonovski in Robinson (3)], was proposed by Hootele (4) and independently supported by a ^{1}H-nmr analysis using nOe (5). Total synthesis of (±)-**2** was reported by Shishido *et al.* (6) and by Wright *et al.* (7). For preparing larger quantities of natural **2** needed for our investigations we adopted the procedure of Nakagawa *et al.* (8), using *m*-chloroperbenzoic acid in the oxidation of **1** with isolation of **2** as picrate or formate salts. Base **2**, obtained from the picrate salt as a gum, converted quantitatively into crystalline **1** with Zn in HOAc (9) but showed (as did the formate salt) two spots of different polarity on Si gel plates.

These findings prompted us to reinvestigate the structure of both free bases and salts of geneserine [**2**] and the analogous geneseroline [**4**]. The ^{1}H-nmr data of bases **2** and **4** are consistent with the structure of a *cis*-fused tetrahydro-1,2-oxazine ring as reported earlier (4,5). The spectra of their salts are quite different from those obtained with **2** and **4**. Considerable down field shifts were observed for all protons in rings B and C of the salts (Table 1). The chemical shifts of H-4 (3.0–3.9) and N_β-Me (3.2–3.3) observed for the salts closely resemble the values observed for molecules with the *N*-oxide structure. Proof of the *N*-oxide structure was made by an X-ray analysis of the HCl salt of geneseroline. The absolute configuration of geneseroline HCl [**5**] is shown in Figure 1; a *cis* relationship of 6-Me and the H-2 proton is apparent.

TABLE 1. Partial ^{1}H-nmr Data of Geneserine [**2**] and Geneseroline [**4**] and Their Salts.

Compound[a]	Proton							
	H_2	H_4	H_4	H_5	H_5	Nα-Me	Np-Me	6-Me
2	4.72	2.67	2.50	2.13	1.95	2.87	2.55	1.20
3	5.59	3.89	3.05	2.81	2.34	3.51	3.25	1.64
4	4.37	2.68	2.51	2.12	1.95	2.83	2.56	1.20
5	5.35	3.83	2.99	2.73	2.31	3.39	3.22	1.59

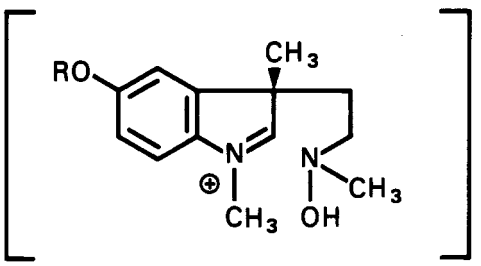
[a]*J* (Hz) of **2**: $J_{4,4'} = 11.7, J_{4,5} = 4.5, J_{4,5'} = 5.0, J_{4',5} = 4.2, J_{4',5'} = 9.4, J_{5,5'} = 14.0$; of **3**: $J_{4,4'} = 11.8$, $J_{4,5} = 5.7$, $J_{4',5} = 13.1$, $J_{4',5'} = 5.3$, $J_{5,5'} = 12.8$, $J_{2,4} = 1.4$; of **4**: $J_{4,4'} = 11.8$, $J_{4,5} = 4.8$, $J_{4,5'} = 4.9, J_{4',5} = 3.9, J_{4',5'} = 9.2, J_{5,5'} = 13.7$; of **5**: $J_{4,4'} = 11.7, J_{4,5} = 5.9, J_{4',5} = 13.0, J_{4',5'} = 5.8$, $J_{5,5'} = 13.0, J_{2,4} = 1.0$.



1

3 R=CONHCH$_3$
5 R=H

2 R=CONHCH$_3$
4 R=H



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A reversible interconversion between the tetrahydro-1,2-oxazine structure (**2**, **4**) and the *N*-oxide structure (**3**, **5**) of geneserine and geneseroline can be shown by monitoring their nmr spectral changes with addition of acid or base. Conversion of **2** and **4** with trifluoroacetic acid into **3** and **5**, respectively, may proceed via a ring-open indolium species **6** already encountered in the conversion of hydroxylaminooxidoles in the total synthesis of (±)-**2** by Shishido *et al.* (6). Base-catalyzed conversion of the *N*-oxide salts **3** and **5** into oxazines **2** and **4**, on the other hand, may be the result of a Meisenheimer type rearrangement already discussed by Nakagawa *et al.* (8), which has also been encountered in the base-catalyzed conversion of 1-benzylisoquinoline *N*-oxides into hydroxylamines (10). The present results, therefore, show that geneserine and geneseroline prefer the tetrahydro-1,2-oxazine structure, while salts of these compounds adopt the *N*-oxide structure.

X-RAY CRYSTALLOGRAPHIC DATA FOR GENESEROLINE HYDROCHLORIDE.—$C_{13}H_{19}N_2O_2^+$ $Cl^-\cdot H_2O$, formula weight = 288.81, monoclinic, space group $P2_1$; $a = 8.325(1)$, $b = 6.967(1)$, $c = 13.079(1)$ Å; $\beta = 96.45°$, $d_{calcd} = 1.27$ g cm^{-3}, $\mu = 2.31$ mm^{-1}, $Z = 2$ (1 molecule/asymmetric unit), 1959 independent reflections (including Friedel equivalents for absolute configuration calculations) were measured out to 2θ max = 115° with a Nicolet R3MV diffractometer using CuKα radiation ($\lambda = 1.548$ Å) with an incident beam graphite monochromator. The data were col-

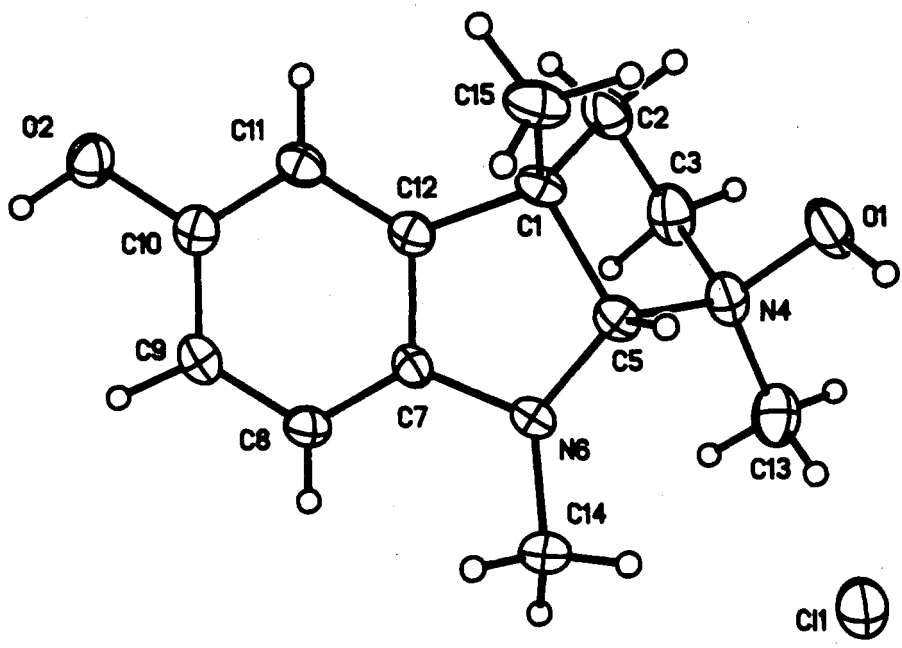

FIGURE 1. Diagram showing the structure and absolute configuration of geneseroline *N*-oxide hydrochloride [**5**·HCl]. The figure is drawn using experimentally determined coordinates with arbitrary thermal parameters.

lected at 295 K from a clear, colorless crystal (0.3 × 0.3 × 0.1 mm) using the θ/2θ scan technique with a variable 2θ scan rate dependent on the intensity of the reflection (10°/min minimum to 30°/min maximum). The structure was solved by direct methods as implemented by the SHELXTL system of programs (11). Least-squares refinement on 238 parameters (coordinates for all atoms, anisotropic thermal parameters for non-hydrogen atoms, isotropic thermal parameters for hydrogen atoms except those on the Me groups, which were placed at calculated positions, C-H = 0.96Å, and allowed to ride on the covalently bonded C atom). The 1918 reflections for which $|F_o| < 3\sigma|F_o|$ gave a final *R*-factor of 0.046 ($R_w = 0.057$). The goodness-of-fit parameter was 2.33, and the final difference map was featureless except for some ripples in the vicinity of the Cl atom. Table 2 lists the coordinates and U_{eq} values for the non-hydrogen atoms.[1]

DISCUSSION OF X-RAY RESULTS.—The results of the X-ray study on geneseroline HCl are illustrated in Figure 1. The absolute configuration (1*S*, 5*S*) was determined from the anomalous scattering by using Friedel pairs as suggested by Rogers (12). The fused five- and six-membered ring system is planar, and the terminal five-membered ring is in an envelope conformation with C-3 being the out-of-plane atom. The two five-membered rings are *cis* fused. The *N*-oxide nitrogen is tetrahedral (average C-N-C and O-N-C angles = 109.4°). There is one molecule of H_2O co-crystallized with the geneseroline HCl which figures prominently in the intermolecular packing by being the donor in two hydrogen bonds and the acceptor in a third one. It acts as a donor to two different (but symmetry-related) Cl atoms with the H_2O–Cl distances being 3.09 and 3.08 Å. The H_2O molecule is the acceptor in a very strong hydrogen bond involv-

[1]Atomic coordinates have been deposited with the Cambridge Crystallographic Data Centre and can be obtained from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

TABLE 2. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for Geneseroline *N*-oxide HCl [**5**].

Atom	*x*	*y*	*z*	U (eq)[a]
Cl-1	740(2)	−12	6386(1)	76(1)
C-1	3492(4)	2836(6)	1913(3)	41(1)
C-2	5259(5)	2240(8)	2307(3)	60(2)
C-3	5062(5)	402(8)	2911(3)	64(2)
N-4	3642(4)	841(5)	3472(2)	50(1)
C-5	2411(4)	1813(6)	2643(3)	42(1)
N-6	1458(3)	435(5)	2061(2)	47(1)
C-7	1758(4)	543(5)	1025(2)	37(1)
C-8	1051(5)	−467(6)	201(3)	47(1)
C-9	1491(4)	−101(6)	−774(3)	49(1)
C-10	2645(4)	1298(6)	−903(3)	45(1)
C-11	3389(4)	2276(6)	−66(3)	45(1)
C-12	2936(4)	1912(6)	902(2)	39(1)
C-13	3015(6)	−856(8)	3997(3)	70(2)
C-14	−147(5)	−64(8)	2318(3)	62(1)
C-15	3246(5)	5023(7)	1896(3)	62(2)
O-1	4183(3)	2240(5)	4213(2)	68(1)
O-2	3079(3)	1748(5)	−1859(2)	56(1)
O-3	1946(4)	3445(6)	5226(2)	65(1)

[a]Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.

ing the hydroxyl group O-1 with the O-1–O (H_2O) distance = 2.55 Å, the H–O (H_2O) distance = 1.48 Å and the O-H–O angle = 172.7°. There is a fourth hydrogen in which the hydroxyl group on O-2 is the donor to a Cl atom (O–Cl = 3.10 Å).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns melting point apparatus, and optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. ^{1}H-nmr spectra were measured on a Varian XL-300 (300 MHz) spectrometer, and chemical shifts are reported in δ with TMS as the internal reference. Mass spectra were taken on a Finnigan 1015 D instrument. Elemental analysis was performed by Microlit Laboratories.

GENESERINE [**2**].—Physostigmine (2 g, 7.26 mmol) was dissolved in $CHCl_3$ (200 ml), and 3-chloroperbenzoic acid (1.8 g, 8.87 mmol of 85% purity) in $CHCl_3$ (20 ml) was added slowly at 0–5°. The reaction mixture was stirred at room temperature overnight. The $CHCl_3$ solution was washed with a saturated aqueous solution of $NaHCO_3$ until the aqueous layer became basic, then washed with brine and dried over $MgSO_4$. After removing the solvent by vacuum, the residue was dissolved in Et_2O and washed with $NaHCO_3$ followed by brine. After drying over $MgSO_4$, evaporation of solvent gave geneserine as a gum (1.7 g, 80.4%): $[\alpha]^{22}D$ −170° (EtOH, c = 0.5) [lit. (1) $[\alpha]D$ −175° (EtOH)]; cims *m/z* $[M+1]^+$ 291; ^{1}H nmr see Table 1.

PICRATE SALT OF PHYSOSTIGMINE *N*-OXIDE.—Compound **3** was prepared from **2** with EtOH picric acid. Yellow crystals, mp 174–176° [lit. (8) mp 174–175.5°]; $[\alpha]^{22}D$ −100° ($CHCl_3$, c = 0.2); ^{1}H nmr see Table 1. *Anal.* calcd for $C_{15}H_{21}N_3O_3 \cdot (O_2N)_3C_6H_2OH$: C 48.45, H 4.84, N 16.15%; found C 48.87, H 4.64, N 16.03.

FORMATE SALT OF PHYSOSTIGMINE *N*-OXIDE.—Prepared from **2** with ethanolic HCO_2H. Colorless crystals; mp 152–154°; $[\alpha]^{22}D$ −170.5° (EtOH, c = 0.2). *Anal.* calcd for $C_{15}H_{21}N_3O_3 \cdot HCOOH \cdot H_2O$: C 54.07, H 7.09, N 11.82; found C 53.74, H 6.90, N 12.34.

REDUCTION OF GENESERINE WITH ZINC.—Geneserine base [**2**] (500 mg), HOAc (2 ml), and Zn powder (100 mg) were stirred at room temperature for 0.5 h. After evaporation in vacuo followed by addition of H_2O (5 ml) and basification with Na_2CO_3, the aqueous phase was extracted with Et_2O to afford, in

quantitative yield, **1**: mp 106–107° [lit. (10), mp 105–106°]; $[\alpha]^{22}D$ −76° ($CHCl_3$, c = 1.0) [lit. (13), $[\alpha]D$ −76° ($CHCl_3$, c = 1.3)].

GENESEROLINE [**4**].—Geneserine (500 mg, 1.72 mmol) was dissolved in *n*-BuOH (25 ml), and a small piece of Na (about 1 mg) was added. The reaction mixture was refluxed for 0.5 h under N_2, then acidified with saturated ethanolic HCl. After evaporation of solvent in vacuo, the residue was dissolved in H_2O and washed with Et_2O. The aqueous solution was made basic with $NaHCO_3$, then extracted with Et_2O. The organic phase was concentrated to give the crystalline base geneseroline [**4**] (230 mg, 57.1%): mp 151–151° [lit. (14) mp 154°]; $[\alpha]^{22}D$ −170° (EtOH, c = 0.5) [lit. (10) $[\alpha]D$ −176° (EtOH)]; cims m/z $[M+1]^+$ 235; ^1H nmr see Table 1.

HYDROCHLORIDE SALT OF GENESEROLINE *N*-OXIDE.—The crystalline base **4** was dissolved in EtOH and acidified with ethanolic HCl. It was left in the refrigerator overnight to give a crystalline hydrochloride salt which was recrystallized from EtOH to give a pure hydrochloride salt of geneseroline *N*-oxide [**5**]: mp 153–155° [lit. (14) mp 154°], $[\alpha]^{22}D$ −229° (EtOH, c = 0.1). *Anal.* calcd for $C_{13}H_{18}N_2O_2 \cdot HCl$: C 57.66, H 7.07, N 10.35; found C 57.39, H 7.07, N 10.26. Hydrochloride **5** could readily be converted into geneseroline [**4**] by dissolution in H_2O and addition of Na_2CO_3.

GENESERINE [**2**] FROM GENESEROLINE [**4**].—Crystalline geneseroline base (650 mg, 0.21 mmol) was dissolved in Et_2O (20 ml), and a small piece of Na (less than 1 mg) was added. After stirring for 2 min, MeNCO (14.6 mg, 0.26 mmol) was added to the reaction mixture slowly and dropwise (by microsyringe). Immediate evaporation of solvent under vacuum gave a residue which was dissolved in Et_2O, washed with saturated aqueous solution of $NaHCO_3$ followed by brine, and dried with $MgSO_4$. Evaporation of Et_2O gave geneserine as a gum (50 mg, 82°%). Tlc, $[\alpha]^{22}D$, and ms were identical with a sample from physostigmine by oxidation. The picrate salt prepared was identical with a reference sample.

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