Nortropacocaine Hydrochloride Conformation in Aqueous and Hydrophobic Media

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Abstract □ The nortropacocaine hydrochloride PMR spectra in deuterium oxide and in deuterochloroform differed markedly. A detailed conformational analysis using vicinal ^1H-^1H coupling constants revealed the molecular conformation to be identical in both solvents. The preferred conformation was one in which the piperidine component existed as a deformed chair. The spectral differences were due to a decreased deshielding of the protonated nitrogen on the neighboring bicyclic ring protons, resulting in chemical shift changes.

Keyphrases □ Nortropacocaine—conformation, effect of solvents, PMR spectra, structure-activity relationships □ Cocaine analogs—nortropacocaine, conformation, structure-activity relationships, effect of solvents, PMR spectra □ Anesthetics, local—nortropacocaine, conformation, structure-activity relationships □ Stimulants, central—nortropacocaine, conformation, structure-activity relationships

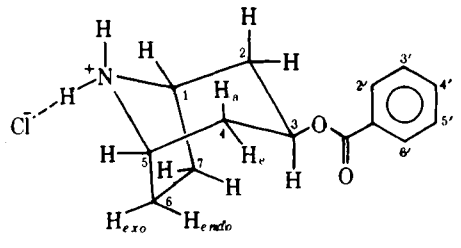
Cocaine, the most prominent tropane alkaloid obtained from *Erythroxylon coca* leaves, possesses dual pharmacological activity (1). Systemic administration interferes with the uptake of catecholamines by adrenergic nerve terminals, and local application blocks nerve conduction. Therefore, the drug has central nervous system stimulant as well as local anesthetic properties.

One explanation for this dual activity is that cocaine interacts in two different conformations (2, 3). The catecholamine uptake inhibition is associated with a noncontiguous transoid phenethylamine cocaine conformation (2) while the local anesthetic activity is associated with a piperidine boat conformation (3). This hypothesis is similar to that of Schueler (4), according to which acetylcholine in a "cisoid" conformation stimulates the nicotinic receptors while acetylcholine in a "transoid" conformation stimulates the muscarinic receptors.

BACKGROUND

Although examples of 4-piperidinols in boat conformations have been reported (5, 6), no tropane analogs have been shown to possess such a conformation. X-ray diffraction studies on salts of cocaine (7) and its analogs (8) provided no evidence for a piperidine boat. Similarly, cocaine free base was found in a conformation having the piperidine ring in a chair form. However, a boat conformation was reported (9) to be a necessary intermediate during the reversible acid-catalyzed N → O acyl migration in *N*-benzoylnortropine and several of its analogs.

This study investigated the conformation of *O*-benzoylnortropine (nortropacocaine) (Structure I) hydrochloride, a monosubstituted tropane analog resembling cocaine only in those structural features believed to favor a piperidine boat conformation (Structure II). Such a boat conformation would be stabilized through an interaction—electrostatic or hydrogen bonding—between the ^+NH group and the *O*-benzoyloxy oxygen.



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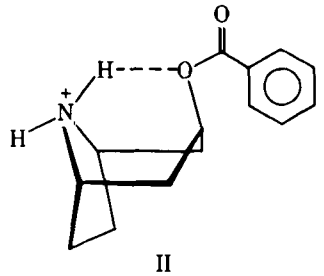

II

Conformational analyses of several tropane alkaloid free bases in deuterochloroform (8) and of tropane salts in deuterium oxide (10–12) have been carried out from their PMR spectra. However, the conformations of tropane salts in hydrophobic media have not been investigated. The present study represents a detailed conformational analysis of a cocaine analog salt in aqueous and in hydrophobic media, with special emphasis on the possible role of the solvent. This study is also the first conformational analysis of a tropane salt in hydrophobic media.

Solvent changes have been reported to produce pronounced effects on some molecular conformations (6, 13). To observe any such solvent effects, the nortropacocaine hydrochloride conformation was determined in deuterium oxide and in deuterochloroform using high-resolution PMR. Nonpolar solvents, in addition to representing more accurately the nerve membrane hydrophobic environment, should favor the boat conformation by enhancing the H_2N^+–O interaction. This study is further justified by the great interest in tropane analogs as models of various drug pharmacophoric conformations (14).

EXPERIMENTAL

The ^1H resonance spectra were measured at 270 MHz (Figs. 1*a* and 1*b*) using 0.10 *M* solutions in deuterochloroform and in deuterium oxide at an ambient temperature of 20°. Chemical shifts were measured from tetramethylsilane and sodium 2,2-dimethyl-2-silatane-5-sulfonate, which were used as internal standards. Nortropacocaine hydrochloride (9) was obtained by *N*-demethylating tropacocaine using 2,2,2-trichloroethylchloroformate (15). Tropacocaine hydrochloride was obtained commercially.

All of the ^1H chemical shifts and ^1H-^1H coupling constants for the protons in the piperidine component of the tropane nucleus could be

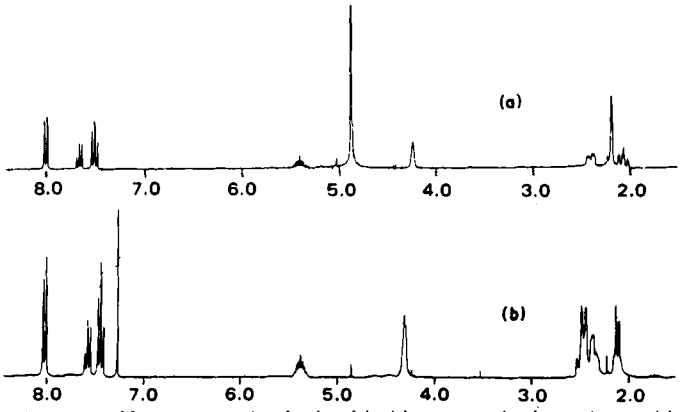

Figure 1—*Nortropacocaine hydrochloride spectra in deuterium oxide* (a) *and in deuterochloroform* (b).

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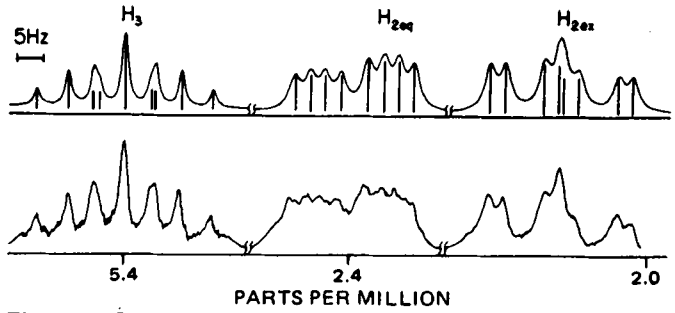


Figure 2—*Real (bottom) and simulated (top) spectral regions from the ^1H-nortropacocaine hydrochloride spectrum in deuterium oxide.*

extracted by analyzing the spectral portions due to the methine proton on C_3 and the methylene protons on C_2. The absorption signals due to the H_3 methine proton were analyzed as the *C* portion of an *AA'BB'C* system. Signals due to each geminal proton on C_2 were sufficiently separated to allow their analysis as the *AB* portion of an *ABCD* system. In the preliminary analysis, the *AA'BB'C* and *ABCD* spectra were correspondingly approximated as $(AB)_2X$ and *ABKY* systems. Initial spectral parameter estimates were obtained by standard methods (16) with the aid of ^1H-^1H spin decoupling, when necessary, and were then refined by spectral simulation and iteration using the ITRCAL program. This program is an implementation of the LAOCN 3 algorithm (17) on a minicomputer. It requires a 12K computer system and a disk memory. Experimental and "best-fit" simulated spectra are shown in Figs. 2 and 3.

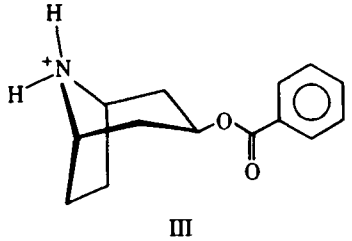
Due to overlap, the spectral portions corresponding to the protons on C_6 and C_7 of the ethylene bridge were not amenable to detailed analysis. Only approximate individual chemical shifts could be extracted directly from the spectra with the help of ^1H-^1H spin decoupling.

RESULTS AND DISCUSSION

The nortropacocaine hydrochloride spectra in deuterium oxide (Figs. 1*a* and 2) and in deuterochloroform (Figs. 1*b* and 3) had similar H_3 proton resonances but varied markedly in other regions. Spectral assignments in the crucial $H_{2,4}$ region (Figs. 2 and 3) could be made only after ^1H-^1H spin decoupling. The variations between the two spectra could be attributed to differences in coupling constants, chemical shifts, or both.

Coupling Constants—The preferred molecular conformation could be determined unambiguously from the piperidine ring proton coupling constants. In both solvents, the coupling constants showed no appreciable differences, indicating that no significant conformational changes occurred when an aqueous medium was substituted for a hydrophobic solvent. The most direct information on this tropane analog conformation came from the C_3 proton signal. The coupling constants ($J_{2ax,3}$ and $J_{2eq,3}$) between this proton and its neighboring methylene protons are consistent with H_{ax}-H_{ax} and H_{ax}-H_{eq} coupling, indicating that the piperidine ring exists as a slightly distorted chair (Structure III). In this chair, the H_3 proton occupies an axial position. Consequently, the benzoyloxy group is equatorial. The $J_{2eq,3}$ and $J_{2ax,3}$ were assumed to have like signs and to be positive since vicinal couplings in substituted ethanes have the same sign and are regarded as positive (18). The $J_{2ax,2eq}$ was assumed to be negative since the geminal proton coupling is negative for most CH_2 groups (18). Additional evidence for a distorted piperidine chair conformation was provided by the coupling constants ($J_{1,2ax}$ and $J_{1,2eq}$) of the C_2 protons with the C_1 bridge carbon proton.

If nortropacocaine hydrochloride is assumed to exist as a single conformer in solution, the piperidine ring distortion can be evaluated by comparing the vicinal ^1H-^1H coupling constants with the coupling constants calculated for a "perfect" chair. This procedure, which makes use of substituent electronegativities (19), was developed (20) for perfectly staggered 1,2-disubstituted ethanes and has given good results in the past



III

Table I—^1H Vicinal Coupling Constants for Nortropacocaine Hydrochloride

Constant	Deuterochloroform	Deuterium Oxide	Calculated
$J_{1,2eq}$	2.9	3.25	2.37
$J_{1,2ax}$	2.35	2.85	3.63
$J_{2eq,3}$	5.8	5.75	5.19
$J_{2ax,3}$	10.8	10.75	12.70
$J_{2ax,2eq}$	−13.95	−13.85	—

(21). First, coupling constants for a perfectly staggered piperidine ring were calculated (20). The values thus obtained were compared to the experimentally determined coupling constants of the piperidine ring in nortropacocaine hydrochloride (Table I), and the following dihedral angles were calculated for nortropacocaine hydrochloride in deuterochloroform and in deuterium oxide, respectively: $\phi(H_1C_1C_1$-$H_{2ax}) = 66$ and 64°, $\phi(H_1C_1C_2H_{2eq}) = 56$ and 54°, $\phi(H_{2ax}C_2C_3H_{3ax}) = 158$ and 157°, and $\phi(H_{2eq}C_2C_3H_3) = 58$ and 58°.

Because of the dangers involved in using quantitative correlations to obtain precise dihedral angles, such values are only approximate. The dihedral angles deviations from ideality were compatible with a piperidine ring slightly flattened at the C_3 position and more puckered at the $^+NH_2$ group (Structure III).

Similar conclusions about the piperidine ring distortion were reached using a method (22, 23) for the qualitative determination of six-membered ring distortions from the ratios of J_{trans}-J_{cis} vicinal coupling constants.

Chemical Shifts—The pronounced changes that the nortropacocaine hydrochloride proton spectrum underwent when the solvent was changed from deuterochloroform to deuterium oxide were due almost exclusively to different individual proton chemical shift changes. The variation in these chemical shift changes could be attributed to a reduction in the protonated nitrogen deshielding influence on the neighboring protons in the bicyclic ring when deuterochloroform was replaced with deuterium oxide. Such solvent modification of the charged nitrogen deshielding influence was demonstrated elsewhere (24). The highly polar deuterium oxide presumably (24) decreases the effect of the positive charge through direct solvation of the positive ion. The result is that in deuterium oxide the protons in close proximity to the protonated nitrogen are shifted upfield relative to the corresponding protons in the less polar deuterochloroform.

An attempt was made to relate the chemical shift differences in the

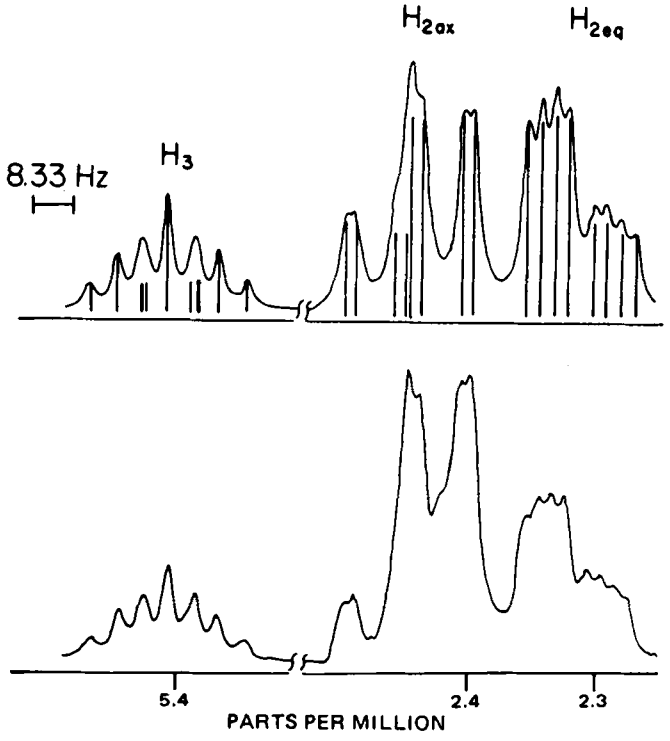


Figure 3—*Real (bottom) and simulated (top) spectral regions from the ^1H-nortropacocaine hydrochloride spectrum in deuterochloroform. In the real spectrum, H_{2ax} and H_7 protons are overlapping.*

Table II—^{1}H Chemical Shifts for Nortropacocaine Hydrochloride (270 MHz)

Proton	Chemical Shift, ppm		Difference, Hz
	Deuterochloroform	Deuterium Oxide	
$H_{1,5}$	4.29	4.24	14
$H_{2eq,4eq}$	2.31	2.39	−22
$H_{2ax,4ax}$	2.44	2.06	103
H_3	5.37	5.40	−8
$H_{6endo,7endo}$	2.07	2.18	−30
$H_{6exo,7exo}$	2.42	2.18	65
$H_{2',6'}$	8.08	8.04	11
$H_{3',5'}$	7.45	7.54	−24
$H_{4'}$	7.59	7.70	−30

two solvents to the relative distances between the protonated nitrogen and the neighboring protons based on the described conformation. Examination of the chemical shift differences (Table II) revealed that the $H_{2ax,4ax}$ as well as the $H_{6exo,7exo}$ protons that showed large downfield shifts lay in close proximity to the positively charged nitrogen. On the other hand, the $H_{2eq,4eq}$, $H_{6endo,7endo}$, and H_3 protons that were farthest removed from the nitrogen showed the small upfield shifts generally observed when the solvent was changed from deuterium oxide to deuterochloroform. On the whole, the correlation between chemical shift differences and nitrogen–proton distances was very good and supported the conformational assignments.

The chemical shift changes on the phenyl ring protons could be explained by using a similar argument. The carbonyl group contributes to the deshielding of the ring protons, which lie in the same plane with it (25). The diminished deshielding resulting from carbonyl group solvation when deuterium oxide was used as a solvent was more pronounced on the nearest *ortho*-protons and decreased progressively with the *meta*- and *para*-protons.

Reports of changes in the proton chemical shifts following a solvent change were interpreted as a conformational change in the molecule. The results reported here point out that conformational interpretations based solely on changes in chemical shifts should be made cautiously. Preferably, such results should be supplemented with coupling constant measurements, still the most reliable information source on the conformations of small molecules in solution.

Failure to demonstrate significant quantities of a piperidine boat conformation in this cocaine-like molecule in solution indicated that such a species is unstable and suggested that it does not participate in events leading to local anesthesia. The occurrence of a chair to boat conformational change when the molecule comes in contact with the nerve membrane could not, however, be excluded. The conformation of cocaine and its analogs in the presence of membrane preparations is currently under investigation.

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