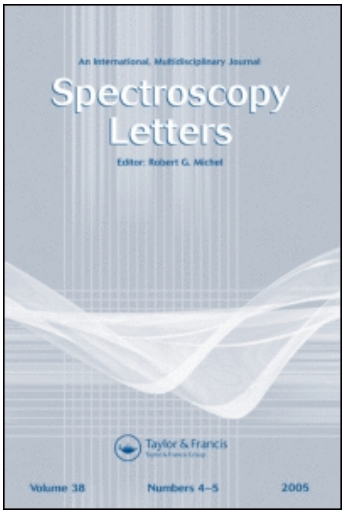


Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Identification of a Heroin Diluent: One- and Two-Dimensional Proton and Carbon-13 NMR Studies of Procaine Hydrochloride: Computational Studies of Procaine and Its Conjugate Acid

Robert Rothchild[a]
[a] Science Department, John Jay College of Criminal Justice, The City University of New York, New York, NY, USA

Online publication date: 29 April 2003

To cite this Article Rothchild, Robert(2003) 'Identification of a Heroin Diluent: One- and Two-Dimensional Proton and Carbon-13 NMR Studies of Procaine Hydrochloride: Computational Studies of Procaine and Its Conjugate Acid', Spectroscopy Letters, 36: 1, 35 — 42
To link to this Article: DOI: 10.1081/SL-120021171
URL: http://dx.doi.org/10.1081/SL-120021171

SPECTROSCOPY LETTERS
Vol. 36, Nos. 1 & 2, pp. 35–42, 2003

Identification of a Heroin Diluent: One- and Two-Dimensional Proton and Carbon-13 NMR Studies of Procaine Hydrochloride: Computational Studies of Procaine and Its Conjugate Acid

Robert Rothchild*

The City University of New York, John Jay College of Criminal Justice, Science Department, New York, New York, USA

ABSTRACT

An unknown powder was recently acquired by street ethnographers from a distributor who routinely ''cut'', adulterated and diluted, his heroin with this product. We present here the results of one- and two-dimensional (1D and 2D) proton and carbon-13 NMR studies of this material that resulted in its identification as procaine hydrochloride. In addition, we give the results of ab initio molecular modeling calculations

*Correspondence: Robert Rothchild, The City University of New York, John Jay College of Criminal Justice, Science Department, 445 West 59th St., New York, NY 10019–1128, USA; E-mail: rrothchild@jjay.cuny.edu.

DOI: 10.1081/SL-120021171 0038-7010 (Print); 1532-2289 (Online)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

(Hartree-Fock level, 6-31G* basis set) for both procaine free base and for its protonated conjugate acid.

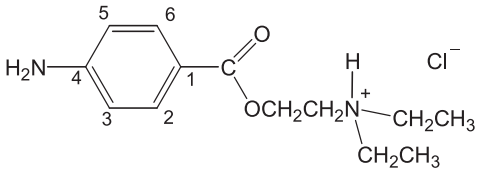
Key Words: Procaine; 1D and 2D NMR; Analysis; Molecular modeling; Ab initio; Heroin.

INTRODUCTION

A recent case study by John Jay College investigators[1] involved the examination of an unidentified powder, allegedly ''morphine,'' that was routinely used as a diluent or adulterant by a long time heroin dealer in New York City. That study primarily treated the public health implications of the adulteration of illegal drugs. In this present report, we describe the detailed results of one- and two-dimensional (1D and 2D) proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopic studies of the unidentified powder. These NMR results allowed us to identify the sample as procaine hydrochloride, *1*, of high purity. The formal structure (and alternative names) of *1* are shown in Scheme 1. To complement these findings, we have also performed ab initio geometry optimizations for both procaine free base, *2*, and for the protonated (conjugate acid) procaine.

EXPERIMENTAL

All spectra were acquired at ambient temperatures in 5 mm sample tubes with a Bruker ACF300 NMR spectrometer (7 Tesla) equipped with



PROCAINE HYDROCHLORIDE

4-AMINOBENZOIC ACID 2-(DIETHYLAMINO)ETHYL ESTER HYDROCHLORIDE

2-DIETHYLAMINOETHYL *p*-AMINOBENZOATE HYDROCHLORIDE

CAS Registry No. [51-05-8]

Scheme 1.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Aspect A3000 data system and QNP quad nuclear probe. Observation frequencies were 300 MHz for proton and 75 MHz for carbon-13, and standard Bruker software and microprograms were applied. Solvent d_6-dimethyl sulfoxide (99.9 atom % D) was obtained from Aldrich Chemical (Milwaukee, WI) and was stored over 3A Molecular Sieves. Chemical shifts are reported in parts per million (ppm) relative to internal tetramethylsilane at 0.0 ppm. Relative peak areas are expressed as 1H, 2H, 3H, etc., with multiplicities given as s (singlet), d (doublet), t (triplet) or q (quartet), and the observed splittings are given in Hz.

Molecular modeling calculations were initially performed using PC Spartan, version 1.4c (Wavefunction, Inc., Irvine, CA, 1996–1997) on a Gateway 2000 Pentium Pro platform with 200 MHz processor speed and 64 MB RAM. Calculations were subsequently carried out using TITAN, version 1.0.5 (Wavefunction, Inc., 1999) on a Dell Pentium 3 PC with 1.2 GHz processor speed and 512 MB RAM. Qualitatively, the latter system seemed at least ten times faster, with optimizations typically achieved in one or two days which had taken more than a month on the earlier system.

DISCUSSION AND RESULTS

In the context of illegal ''street drugs,'' we may consider ''adulterants'' as added substances that themselves have significant pharmacological activity, e.g., other illegal drugs or pharmaceuticals. ''Diluents'' may refer to substances added simply as fillers or extenders of various types with no significant pharmacological properties. The presence of different adulterants and diluents, and the patterns of their use, may have much importance as a public health issue as well as being a law enforcement issue. The added substances may have interactions or synergistic effects with one another as well as with the primary illicit substance in a sample. Such interactions can have potential significance with respect to both acute and chronic toxicity of the street samples. An understanding of the usage of various adulterants and diluents can therefore be of much importance.[1]

Confirmation of the procaine structure of the initially unknown sample was obtained by a series of one- and two-dimensional (1D and 2D) nuclear magnetic resonance (NMR) spectroscopy experiments. (NMR data are taken from Ref. [1].) Thus, a solution of the unknown sample (6.0 mg in 468 mg d_6-dimethyl sulfoxide, DMSO) showed a remarkably clean 1D proton NMR spectrum. Spectral data of significant absorption peaks are as follows, and proposed assignments are shown in parentheses. Proton NMR: 10.75 ppm, 1H, broad s, (HN+); 7.69 ppm, 2H, d, 8.56 Hz (aryl H-2,6); 6.59 ppm, 2H, d, 8.59 Hz (aryl H-3,5); 6.08 ppm, 2H, slightly broad s (NH_2); 4.53 ppm,

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

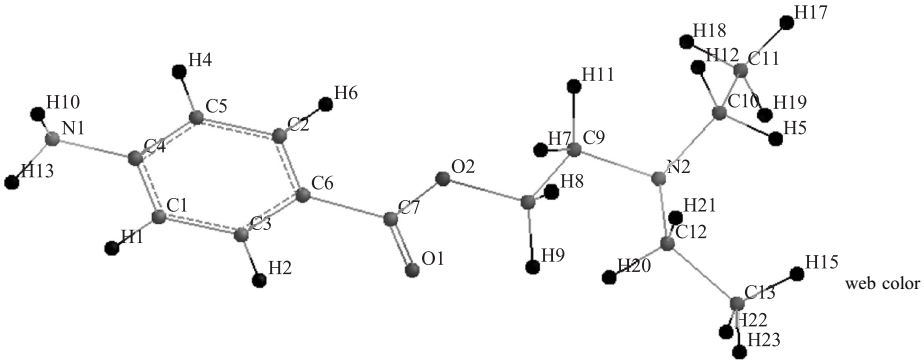
2H, slightly broad t, 4.99 Hz (OCH_2); 3.45 ppm, 2H, highly broadened t (see text) (OCH_2CH_2N+); 3.19 ppm, 4H, slightly broad q, 6.94 Hz (NCH_2CH_3); 1.25 ppm, 6H, t, 7.20 Hz (CH_3). The broad 1H singlet is consistent with the single hydrogen on the ammonium nitrogen, deshielded by the formal charge on the N, with broadening due to chemical exchange and quadrupolar broadening by nitrogen. The two sets of aromatic protons appear as an ''AB quartet'' (actually AA′BB′), consistent with the 1,4-disubstituted benzene ring. The coupling constant magnitude reflects typical values, and the equality of the coupling constants for each doublet (together with the ''leaning'' of the doublets) support mutual splitting. The lower field doublet is assigned to H-2,6, *ortho* to the ester; the higher field doublet would correspond to H-3,5, shielded by their *ortho* relationship to the mesomerically electron-releasing amino group. The slightly broad singlet of the aryl NH_2 appears at 6.08 ppm. The CH_2CH_2 moiety appears as two broadened triplets. The lower field triplet, centered at 4.53 ppm, deshielded by oxygen, is only slightly broadened, with distinct valleys between the three branches. The more profoundly broadened ''triplet'' at 3.45 ppm shows only shoulders and no valleys (local minima) to imply triplet character. The more severe broadening may be attributed to chemical exchange (and nitrogen quadrupolar broadening) of the $NH+$, and allows assignment of the 3.45 ppm signal to the CH_2NH+, and the sharper and lower field triplet to the OCH_2. At higher field are seen the resonances of the two ethyl groups. Broadening of the quartet is consistent with CH_3CH_2NH+, associated with NH chemical exchange and nitrogen quadrupolar broadening. Differences in observed splittings in the 4H quartet and 6H triplet signals of the ethyl groups are artifactual, resulting from broadening of the quartet.

The 1D carbon-13 spectrum was acquired with 2529 transients and a 2 sec relaxation delay, with significant signals at (ppm): 165.31, 153.81, 131.30, 114.85, 112.56, 58.18, 49.30, 46.85, 8.42. In addition, spectra were acquired using distortionless enhancement by polarization transfer (DEPT45) to distinguish the protonated carbons; the two-dimensional heteronuclear chemical shift correlation spectrum (XHCORR) was acquired to rigorously assign the protonated carbons. For the XHCORR experiment, 256 transients were obtained for each of 128 increments in the t1 (proton) dimension, zero-filling twice in the t1 dimension for a final data matrix of 512×4 K. Six distinct crosspeaks were seen in the XHCORR spectrum, and these correlations permit assignments as follows, for C-13 shifts at: 131.30 ppm (aryl C-2,6); 112.56 (aryl C-3,5); 58.18 (OCH_2); 49.30 (OCH_2CH_2); 46.85 (CH_3CH_2NH+); 8.42 (CH_3). Non-protonated carbons were tentatively assigned based on chemical shift arguments and aromatic substituent chemical shifts, as follows: 165.31 (C═O), 153.81 (aryl C-4), 114.85 (aryl C-1); note that the aryl NH_2 is strongly deshielding for the *ipso*

carbon (C-4) and shielding for the carbons *ortho* or *para* (C-3,5 or C-1) to the NH_2. All chemical shifts and observed splittings appear consistent with standard tabulated values.[2–4] Finally, we have compared our proton and carbon-13 NMR data to published spectra for procaine hydrochloride, *1*, in d_6-DMSO.[5] Excellent agreement is seen for the proton spectrum, with slight differences in shifts or broadening that most likely reflect concentration-dependent effects. Thus, our sample appears to have been more dilute than that in the ''Aldrich Library,'' so that our proton signal for NH+ appeared ca. 0.4 ppm at higher field, and the NH_2 signal ca. 0.09 ppm at higher field. Less broadening was seen for the NCH_2CH_3 proton resonance in our present work. For the C-13 spectrum, every one of the observed shifts in our present work appeared consistently at ca. 0.7 ppm to higher field than those of the ''Aldrich Library.'' We feel that this is an artifact of sample concentration dependence,[3,4] and we believe that our NMR results have rigorously demonstrated the identity of the procaine salt sample. The potential sociological implications of these adulterants or diluents in street drugs, like heroin, were extensively discussed.[1] The full rationale for the 1D and 2D NMR proton and carbon-13 assignments of *1* do not appear to have been previously presented.

The structure of the procaine moiety has been of considerable interest. The X-ray crystal structures for the 1:1 procaine-bis-*p*-nitrophenyl phosphate complex[6] and for procaine hydrochloride, *1*, itself[7] have been published. Structures for procaine penicillin G monohydrate[8] and procaine benzylpenicillin monohydrate[9] have also been reported. Very recently, aqueous solution structures and related properties of *1* in the presence of *beta*-cyclodextrin have been extensively examined using NMR and other techniques, including molecular mechanics calculations with the MM3* force field and the GB/SA solvent model for water.[10] However, our present ab initio HF/6-31G* calculations appear to be the highest level that have been applied to either procaine free base or its protonated form.[11–13] The polarization basis set of 6-31G* uses one Slater orbital expanded in a series of six Gaussian functions for the core electrons, and two Slater orbitals for the valence electrons, with one expanded in a series of three Gaussian functions and one approximated by one Gaussian function. In addition, it sets d-orbitals on the non-hydrogen atoms.

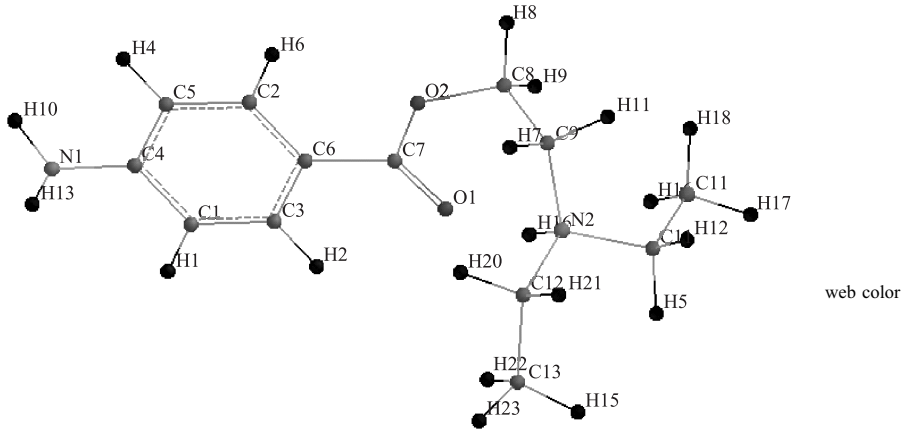
Ab initio molecular modeling calculations were performed in this present work for geometry optimizations at the Hartree-Fock level using the 6-31G* basis set for both the procaine free base, *2*, and for the conjugate acid, i.e., the protonated *2*-H^+. Figure 1 shows the optimized structure and atom numbering for *2*. The lowest energy structure (-762.56539 hartrees) calculated for procaine free base exhibited a relatively extended sidechain structure. For the dihedral angles, C7–O2–C8–C9 and O2–C8–C9–N2,



web color

Figure 1. The optimized structure for Procaine free base, *2*, showing the results of the HF/6-31G* calculation, with the atom numbering.

we calculated values of $-177.8°$ and $+171.7°$, respectively, showing near coplanarity of these atoms. For the protonated procaine, the optimized structure and atom numbering are given in Figure 2. Our calculated results for the protonated procaine imply a structure rather different than that reported for the procaine hydrochloride crystal.[7] The X-ray structure indicated a dihedral angle for C7–O2–C8–C9 of $+172.8°$, nearly anti periplanar, but our calculated structure gave a corresponding angle of $-86.9°$. For the dihedral angle, O2–C8–C9–N2, the X-ray structure of the procaine hydrochloride crystal gave a value of $+70.4°$ versus our



web color

Figure 2. The optimized structure for protonated Procaine, *2*-H+, showing the results of the HF/6-31G* calculation, with the atom numbering.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

calculated value of +74.1°. The key difference accounting for this may reside in our calculated structure providing a strong intramolecular hydrogen bond between the carbonyl oxygen and the proton on the NH+. A distance of 1.789Å was calculated for the O-to-H distance of the C═O···HN+ moiety. In the crystal, the reported X-ray structure showed a nearly tetrahedral NH+ nitrogen with the proton actually directed away from the ester group, making intramolecular H-bonding impossible. Our optimized structure had an energy of −762.97261 hartrees. An alternative minimized structure which was more similar to the X-ray structure of *1* had dihedral angles of +178.4° for C7–O2–C8–C9 and +72.0° for O2–C8–C9–N2, but yielded a much higher energy of −762.95566 hartrees, i.e., 10.6 kcal/mol worse; this higher energy conformation had the NH+ proton directed away from the ester.

CONCLUSIONS

We have presented the detailed proton and carbon-13 NMR characterization of an ''unknown'' material, alleged to be morphine, that had been used as a diluent or adulterant for street samples of heroin in the New York City area. The material was rigorously shown to be high purity procaine hydrochloride. Assignments for the protonated carbons were made based on the 2D heteronuclear chemical shift correlation spectrum, XHCORR. High level ab initio molecular modeling calculations (Hartree-Fock level, 6-31G* basis set) have been performed for both procaine free base and its protonated conjugate acid, and selected important geometric parameters have been discussed. (Detailed listings of the geometric parameters will be available on request from the author.)

ACKNOWLEDGMENTS

Partial support has been provided by grants from the National Science Foundation, U. S. Education Department, and the Professional Staff Congress—City University of New York Research Award Program.

REFERENCES

1. Wendel, T.; Rothchild, R.; Curtis, R.; Corcoran, K.A.; Hamlin, T.; Eng, B.; Zedeck, M.S. Heroin cut with morphine?: an ethnographic/forensic chemistry case study. Addict. Res. Theory. **2003**, *11*, (5). For the Journal's website, see: http://www.tandf.co.uk.

2. Abraham, R.J.; Fisher, J.; Loftus, P. *Introduction to NMR Spectroscopy*; John Wiley: New York, 1988.
3. Günther, H. *NMR Spectroscopy*, 2nd Ed.; John Wiley: New York, 1995.
4. Silverstein, R.M.; Bassler, G.C.; Morrill, T.C. *Spectrometric Identification of Organic Compounds*, 5th Ed.; John Wiley: New York, 1991.
5. Pouchert, C.J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H FT-NMR Spectra*; Aldrich Chem. Milwaukee, 1992; Vol. 1 (2), 1265B.
6. Sax, M.; Pletcher, J.; Gustaffson, B. The crystal structure of a 1:1 procaine-bis-*p*-nitrophenyl phosphate complex. Acta Cryst. **1970**, *B26* (2), 114–124.
7. Beall, R.; Herdklotz, J.; Sass, R.L. Molecular properties of local anesthetics: the crystal structure of procaine hydrochloride. Biochem. Biophys. Res. Commun. **1970**, *39* (3), 329–334.
8. Dexter, D.D.; van der Veen, J.M. Conformations of penicillin G: crystal structure of procaine penicillin G monohydrate and a refinement of the structure of potassium penicillin G. J. Chem. Soc., Perkin Trans., I **1978**, (3), 185–190.
9. Lowe, P.R.; Schwalbe, C.H. Crystal structure of procaine benzylpenicillin monohydrate. J. Pharm. Pharmacol. **1978**, *30*, (Suppl.), 92P. Presented at the 115th meeting of the British Pharmaceutical Conference (Coventry, Sept. 11–15, 1978).
10. Merino, C.; Junquera, E.; Jiménez-Barbero, J.; Aicart, E. Effect of the presence of β-cyclodextrin on the solution behavior of procaine hydrochloride. Spectroscopic and thermodynamic studies. Langmuir **2000**, *16* (4), 1557–1565.
11. Jensen, F. *Introduction to Computational Chemistry*; John Wiley: New York, 1999.
12. *TITAN Tutorial and User's Guide*; Wavefunction, Inc.: Irvine, CA; Schrödinger, Inc.: Portland, OR, 1999.
13. Hehre, W.J.; Shusterman, A.J.; Nelson, J.E. *The Molecular Modeling Workbook for Organic Chemistry*; Wavefunction, Inc. Irvine, CA, 1998.

Received September 30, 2002
Accepted February 16, 2003