Tetrahedron. Vol. 28, pp 2691 to 2696. Pergamon Press 1972. Printed in Great Britain

CARBON-13 NUCLEAR MAGNETIC RESONANCE OF PHARMACEUTICAL AGENTS: BENZOCAINE HYDROCHLORIDE ANESTHETICS

O. A. GANSOW, W. M. BECKENBAUGH and R. L. SASS
Department of Chemistry, Rice University, Houston, Texas 77001

(*Received in the USA* 12 *November* 1971; *Received in the UK for publication* 26 *January* 1972)

Abstract—Carbon-13 nuclear magnetic resonance (CMR) spectra of eleven aromatically substituted benzocaine hydrochloride anesthetics have been measured. Ester carbonyl chemical shifts correlate well with measured carbonyl stretching frequencies and substituent Taft σ_I parameter. Ring substituents show a large effect upon the *para* carbon CMR chemical shifts which correlate well with Hammett σ's, however no measurable effect on the CMR chemical shifts of the carbonyl bonded diethylaminoethylene portion of the molecule is noted. This evidence suggests that interaction of the polar carbonyl group or the aromatic π system may be important in explaining drug potency in *in vivo* systems by modifying the configuration of the anesthetic at the receptor site.

INTRODUCTION

WE WISH TO REPORT the first carbon-13 nuclear magnetic resonance (CMR) study of the medicinally important benzocaine hydrochloride series of local anesthetics. Previous workers have investigated the molecular structure of these procaines by a variety of physical methods including IR[1] and X-ray[2–5] spectroscopy. These studies in conjunction with pharmacological research have attempted to relate electronic and physical structure to physiological activity for *in vivo* systems.

Local anesthetic activity has been correlated with such various different factors as lipid solubility,[6,7] carbonyl bond orders[1] and conformations of the N^+C-C-O backbone[3] (see structure in the Table). Since ^{13}C NMR has been shown to be a sensitive probe of electron density for both aromatic and carbonyl carbons,[8–11] we have determined ^{13}C NMR spectra of eleven benzocaine anesthetics to deduce correlations among substituent electronic (Taft σ_I, σ_R) parameters, carbonyl stretching frequencies (bond orders), molecular structures in solution and anesthetic potencies.

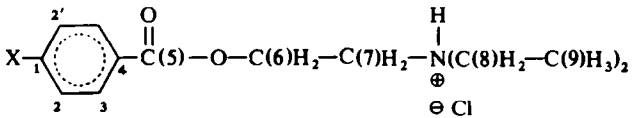
RESULTS AND DISCUSSION

Carbon-13 (CMR) spectra of ten substituted benzocaine hydrochlorides, $[X-C_6H_4-C(O)-OCH_2CH_2N(H)(CH_2CH_3)_2]Cl$, and of procaine amide, $[p\text{-}NH_2-C_6H_4-C(O)N(H)CH_2CH_2-N(H)(CH_2CH_3)]Cl$, were measured versus t-butanol (TBA) internal standard at several pH values throughout the pH region 1–7·4. No variations of shifts with pH were detected until pH 7·4 at which point deprotonation of the amido nitrogen resulted in extensive precipitation. Ester carbonyl IR stretching frequencies were measured in a nujol mull of the salt. Chemical shifts and stretching frequencies together with Hammett and Taft inductive parameters and anesthetic potency data are collected in Table 1.

TABLE 1. COLLECTED DATA INCLUDING CMR CHEMICAL SHIFTS, ν(C=O) cm^{-1}, AND RELATIVE POTENCY DATA.

Subst	1	2	3	4	5	6	7	8	9	σ_{CO} (cm^{-1})[c]	Taft σ_I	σ	Relative[a] potency	Relative[b] potency
p-NH_2	−123·2	84·2	−101·9	−86·8	−137·4	−20·4	28·0	−18·55	21·5	1689	0·10	−0·66	1·0	1·0
p-NH_2 Amide	−125·5	84·9	−99·2	−91·6	−140·7	−5·3	−21·8	−18·3	21·6	1635	0·10	−0·66	..	..
m-Me	−108·7	2 −99·9 2′ −104·5	−98·6	−96·6	−137·2	−20·3	−29·6	−18·7	21·6	1689·5	—	−0·07	—	—
p-EtO	−132·0	−84·4	−101·7	−90·7	−137·2	−20·4	−29·3	−18·6	21·6	1711·6	0·25	−0·25		6·2
p-Me	−115·0	−99·2	−99·2	−95·2	−137·1	−20·2	−29·6	−18·5	21·6	1711·5	−0·05	−0·17	0·80	—
p-H	−104·0	−84·0	−99·4	96·7	−137·2		−29·6	−19·5	21·6	1717·6	0	0	0·85	0·13
p-MeO	−133·8	−84·0	−101·8	−90·9	−136·9	−20·5	−29·3	−18·7	21·5	1703	0·25	−0·27	—	1·25
p-n-BuO	−133·2	−84·5	−101·8	−90·9	−136·3	−20·3	−29·6	−18·6	21·5	1712·5	0·25	−0·25	—	—
p-F	−141·6 −130·4	−86·2 −85·2	−102·7 −102·2	−95·1	−136·2	−20·5	−29·7	−18·7	21·5	1719·5	0·52	+0·06	0·25	—
p-t-Bu	−126·2	−94·6	−99·0	−91·6	−135·7	−19·7	−29·2	−17·9	22·0	1722·5	−0·07	−0·20	—	..
p-Cl	−109·8	−98·7	−101·0	−97·2	−135·9	−20·5	−29·7	−18·6	21·7	1726	0·47	+0·23	0·33	0·83
p-NO_2	−120·4	−93·4	−100·9	−104·3	−135·3	−20·4	−30·4	−18·66	21·7	1727	0·63	+0·78	0·10	0·10

[a] Data from Ref. 20.
[b] Data from Ref. 1.



C-13 chemical shifts in ppm from TBA

Assignment of CMR resonances for a typical benzocaine, *p*-Nitrocaine·HCl was facilitated by reference to CMR spectra of *p*-Fluorocaine·HCl and procaineamide·HCl as follows. Aromatic and carbonyl carbons appear downfield, below −84 ppm vs. TBA. The presence of ^{19}F in *p*-fluorocaine divides each aromatic carbon resonance into a doublet, the magnitude of the C—F doupling constants depending on proximity to fluorine. This has previously been observed for several substituted fluorobenzenes.[22,23] The C-1 carbon resonance is always observed furthest downfield with C-3, C-4 and C-2 signals appearing in that order upfield for all *para*-substituted compounds studied. This quite distinct pattern of resonances is also found for a variety of monosubstituted benzenes.[23]

For the benzocaine carbonyl resonances, CMR shifts range over 5·4 ppm, always downfield from the aromatic carbons. Off resonance decoupling identified the C-9 methyl resonance observed upfield from TBA as well as determining which peaks arise from the three methylene resonances which have intensity ratios of 2 : 1 : 1, the larger signal undoubtedly being derived from the two identical amido methylenes (C-8). Assignment of the C-6, C-7 methylene carbons is achieved by comparison of the spectrum of procaine·HCl to that of procaineamide·HCl. There, replacement of an ester oxygen by nitrogen causes one methylene we assign as C-6 to shift 440Hz upfield while the C-7 methylene shifts only 160 Hz.

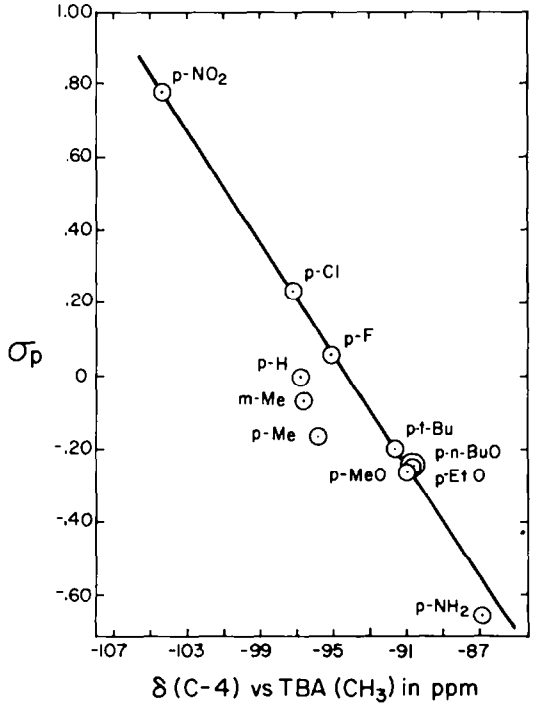


FIG 1. Fraph of Hammett σ_p values vs δ(C-4) in ppm for the benzocaine·hydrochlorides

In several instances,[8,11] it has been demonstrated that CMR signals of *para*-aromatic carbons are sensitive to the electron withdrawing or donating character

of ring substituents. A plot of the Hammett σ_p parameter or of the Taft σ_I parameters of benzocaine against the CMR shift of the aromatic C-4 carbon is shown in the case of the σ_p parameter (Fig. 1) to be linear. It seems clear that electron withdrawal from the ring is reflected by a downfield *para* carbon chemical shift. More interestingly, Fig 2, a plot of the Taft σ_I parameter against the carbonyl chemical shifts, δ(C-5), is also linear. The meaning of this result becomes clear upon consideration of a graph of values for the benzocaine carbonyl stretching frequencies versus C-5 chemical

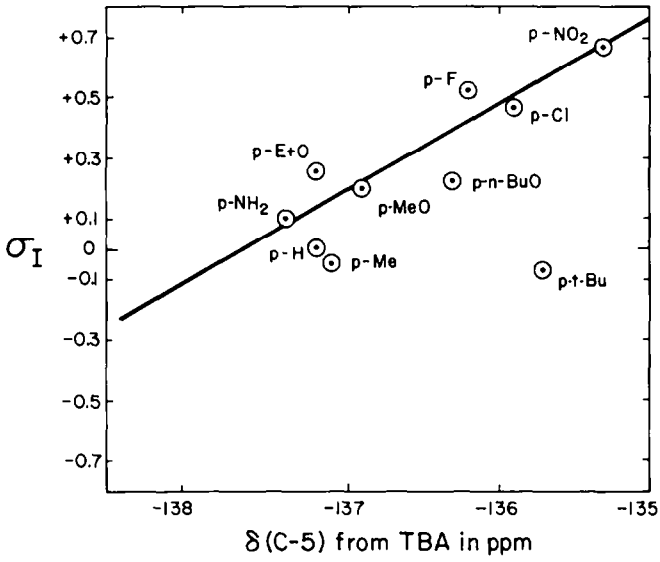


FIG 2. Graph of Taft σ_I values vs δ(C-5) in ppm for benzocaine·hydrochlorides

shifts shown in Fig 3. It is also linear. Maciel[10] has correlated CMR carbonyl shifts of substituted CH_3—CO(X) with carbonyl polarity. The same situation pertains here. As more electron withdrawing groups are substituted in the aromatic ring, the polarity of the CO bond decreases. This correlation is *not* general for aromatic esters. Various authors have in fact noted its absence.[8,13] However, several X-ray structure determinations have shown the benzocaine hydrochloride aromatic ring and carbonyl to be coplanar as required for the efficient conjugation with transmission of ring effects to the carbonyl. This conjugation argues strongly that procaine anesthetics retain co-planarity in solution.

While no specific mechanism has been established for the action of a local anesthetic, it is generally accepted that an interaction between a component of the cellular membrane and the anesthetic is established, perhaps by competition between anesthetic and the structurally similar acetylcholine for binding sites.[5,14–16] Alternatively,[16] the anesthetic may act by inducing conformational changes in proteins.[17] The benzocaines may interact at one or more of three possible binding sites (1) the electropositive quartenary nitrogen; (2) the polar carbonyl function; or (3) a π-complex formation through the aromatic ring.

Some information regarding substituent effects at the possible binding sites for the various benzocaines may be extracted from the ^{13}C NMR chemical shift data. We observe that chemical shifts for the C-6,7,8,9 (the backbone of the anesthetic) vary little for the benzocaines studied. No electronic effects of the substituted benzene are detected beyond the carbonyl functional group, implying that charge on the quartenary nitrogen is invariant. Interactions with the nerve at that position would likely be similar for all benzocaines studied here.

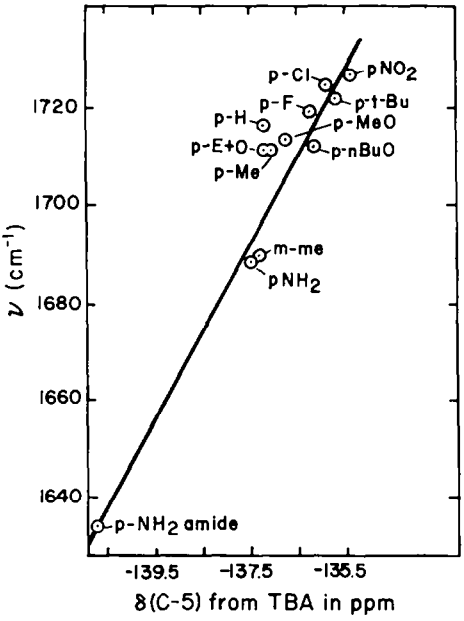


FIG 3. Graph of ν(CO) in cm^{-1} vs $\delta(C^{13}{=}O)$ in ppm for the benzocaine·hydrochlorides

A correlation between carbonyl IR stretching frequencies and anesthetic potency has been suggested by Galinsky *et al.*[1] We note that both IR and carbonyl chemical shift data may be systematically related to the Taft σ_I (inductive) parameter of substituents on the aromatic ring. Withdrawal of electron density decreases carbonyl polarity which may decrease bonding to the nerve substrate. Anesthetic potency does parallel CO polarity. A plot of δ C-5 versus the potency data of Perlia[20] gives a linear correlation of 0·97.

Increased electron density in the π-aromatic system would also strengthen π-complex formation.[19] The ^{13}C shift of the *para* carbon consistently shifts to high field resulting from increased screening due to added π-electron density in the ring. Thyrum *et al.*, have, in fact, detected strong π-complex formation between benzocaines and adenosine triphosphate.[18]

While qualitative agreement is observed between CO polarity, π-complexing ability and anesthetic potency, we must stress that no attempt has been made here to consider other obviously important[6,7] factors on anesthetic action such as lipid solubility.

EXPERIMENTAL

A Brüker HFX-10 spectrometer was employed to obtain CMR spectra. IR spectra were measured in a nujol mull using a Beckman IR-20.

The substituted benzocaine anesthetics were generously supplied by Drs. C. S. Johnson and A. R. Schwartz, Baylor College of Medicine, Houston, Texas. Purity was routinely verified by PMR and IR.

All samples used for CMR work were aqueous solutions *ca.* 0·8 M in the benzocaine with 10% hexafluoroacetone (HFA) added as an internal ^{19}F lock and 5% t-BuOH (TBA) serving as an internal chemical shift references. Resonance shifts are reported in ppm vs. the methyl carbons of TBA. These may be indexed to either $(CH_3)_4$ Si or CS_2 as these resonance are $-29{\cdot}0$ and 164·5 ppm from the methyl TBA signal.

The Brüker spectrometer is equipped with a decoupler useful for both broad band and single resonance proton decoupling. Spectra were obtained using the usual Fourier transform pulse NMR techniques.[12] Free induction decays were time averaged by a Fabri-Tek 1074 computer and Fourier transforms performed on a PDP-8L computer. Usually 1024 averaged pulses were obtained. Shifts were measured accurately to $\pm 0{\cdot}1$ ppm.

Acknowledgement—This research was supported by USPHS Research Grants AM-13704, AM-14305 and HSAA Grant 1-SO4-FR-06136. We acknowledge a Departmental Research Equipment Grant from the National Science Foundation for partial purchase of the Brüker NMR.

REFERENCES

[1] A. M. Galinsky, J. E. Gearien, A. J. Perkins, and S. U. Sustina, *J. Med. Chem.* **6**, 320 (1963)
[2] R. Beall, J. Herdklotz and R. L. Sass, *Biochem. Biophys. Res. Comm.* **39**, 329 (1970)
[4] R. Beall and R. L. Sass, *Ibid.* **40**, 833 (1970)
[4] M. S. Pleichen and B. Gustafson, *Acta Cryst.* **B26**, 114 (1970)
[5] M. B. Feinstein and M. Pamire, *Biochem. Biophys. Acta* **115**, 39 (1966)
[6] H. Vanderhaege, P. Kolosy and M. Claesen, *J. Pharm. Pharmacol.* **6**, 119 (1954)
[7] C. Hansch and T. Fujita, *J. Am. Chem. Soc.* **86**, 1616 (1964)
[8] J. B. Stothers and P. Lauterbur, *Can. J. Chem.* **42**, 1463 (1964)
[9] K. S. Dhami and J. B. Stothers, *Ibid.* **45**, 233 (1966)
[10] G. W. Maciel, *J. Chem. Phys.* **42**, 2746 (1965)
[11] H. Spiesecke and W. H. Schneider, *Ibid.* **35**, 731 (1961)
[12] R. R. Ernst and W. A. Anderson, *Rev. Sci Inst.* **37**, 93 (1966)
[13] J. B. Stothers, *Quart. Rev.* **19**, 144 (1965)
[14] J. M. Ritchie, B. Ritchie and P. Greengard, *J. Pharmacol. Exp. Ther.* **150**, 152 (1965)
[15] L. J. Mullins, *Fed. Proc.* **27**, 898 (1968)
[16] D. Nachmanson, *Chemical and Molecular Basis of Nerve Activity*, Acad. Press, New York (1959)
[17] B. Chance, L. Mela and F. J. Harris, *Fed. Proc.* **27**, 902 (1968)
[18] P. T. Thyrum, P. J. Luchi and H. L. Conn, Jr., *J. Pharmacol. Exp. Ther.* **164**, 239 (1968)
[19] L. J. Andrews, *Chem. Rev.* **54**, 750 (1954)
[20] R. Perlia, *Pharm. Acta Hel.* **42**, 517 (1967)
[21] G. E. Maciel and J. J. Natterstad, *J. Chem. Phys.* **42**, 2427 (1968)
[22] N. Muller and D. Carr, *J. Phys. Chem.* **67**, 112 (1963)