electron-transport chain. *In vivo* studies showed drastic increases in the reduced forms of chain cofactors with either malate or succinate as the substrate. *In vivo* brusatol effects were more striking than *in vitro* effects on reducing cofactors, which may explain the observed increased effects of brusatol on *in vivo* states 4 and 3 respiration after 3 days of treatment as compared to *in vitro* effects. UV *in vitro* studies showed that brusatol chemically interacted with nadide increasing the reduced form absorbance at 340 nm. Cytochrome c, a heme, also was reduced in the presence of brusatol to the ferrous form with an increase in absorbance at 550 nm.

Although separation of the brusatol adduct and reduced nadide has not been achieved to date, incubation of the two agents showed that a possible reaction product existed. Nucleophilic attack of reduced nadide on the diosphenol ring A of brusatol may be postulated. If such an attack occurs, then complexation or alkylation of cofactor or functional groups of enzymes could account for the inhibitory effects of brusatol on P-388 lymphocytic leukemia cell metabolism with respect to the suppression of tumor cell anaerobic and aerobic respiration.

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ACKNOWLEDGMENTS

Adapted in part from a dissertation submitted by S. A. ElGebaly to the Graduate School, University of North Carolina at Chapel Hill, in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported by American Cancer Society Grant CH-19 and National Cancer Institute Grants CA-22929 and CA 17625 (in part) to K. H. Lee.

The authors thank Dr. Matthew Suffness, Developmental Therapeutics Program, Chemotherapy, National Cancer Institute, for the bruceantin.

^{13}C-NMR Spectra of α-Adrenergic Blocking Agents

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Received October 13, 1978, from the *Departments of *Physiology and ‡Chemistry, University of North Dakota, Grand Forks, ND 58202.* Accepted for publication January 17, 1979.

Abstract □ The natural abundance ^{13}C-NMR spectra of five α-adrenergic blocking agents, tolazoline, dibenamine, azapetine, phenoxybenzamine, and phentolamine, are reported. The chemical shifts of various carbon resonances were assigned on the basis of chemical shift theory, multiplicities observed in single-frequency off-resonance-decoupled spectra, relaxation times, and comparisons with the chemical shifts of model compounds.

Keyphrases □ NMR spectroscopy—analysis, tolazoline, dibenamine, azapetine, phenoxybenzamine, phentolamine □ α-Adrenergic blocking agents—analysis, NMR spectroscopy

Earlier studies reporting the ^{13}C-NMR spectra of antimalarials (1, 2), anti-inflammatory agents (3, 4), antipyretic analgesics (5), and central nervous system acting agents (6–10) prompted the assignments of the natural abundance ^{13}C-NMR chemical shifts of α-adrenergic blocking agents, 2-benzyl-2-imidazolone (I) (tolazoline), *N,N*-dibenzyl-β-chloroethylamine (II) (dibenamine), 6,7-dihydro-6-(2-propenyl)-5*H*-dibenz[*c,e*]azepine (III) (azapetine), *N*-(2-chloroethyl)-*N*-(1-methyl-2-phenoxyethyl)benzenemethanamine (IV) (phenoxybenzamine), and 3-[[(4,5-dihydro-1*H*-imidazol-2-yl)methyl](4-methylphenyl)amino]phenol (V) (phentolamine). The ^{13}C-NMR spectra of these therapeutic agents are of theoretical as well as of biological interest.

Both the proton noise-decoupled and single-frequency off-resonance-decoupled (SFORD) spectra of I–V were recorded using the Fourier transform technique. The proton noise-decoupled spectra of these compounds gave the chemical shifts of various carbon resonances, while the SFORD spectra provided the distinction of methyl, methylene, methine, and nonprotonated carbons. Furthermore, the relaxation time measurements differentiated nonprotonated carbons from protonated carbons.

The assignments of various carbon-13 signals were made on the basis of chemical shift theory (11), multiplicities observed in the SFORD spectra, percent integration of the signals in the proton noise-decoupled spectra, relaxation times, and chemical shifts of the corresponding carbons of model compounds (12).

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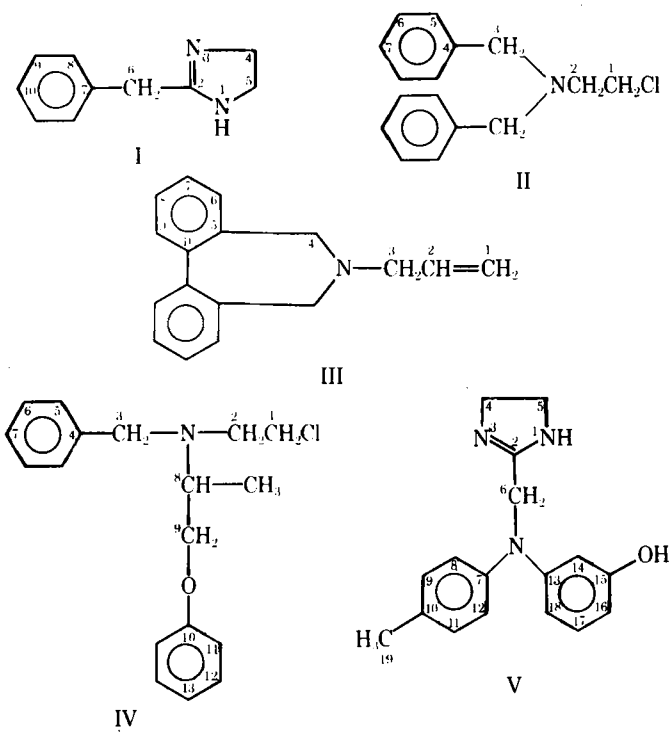


I II III IV V

EXPERIMENTAL

The ^{13}C-NMR spectra of I–V were obtained on a spectrometer[1] operating at 15.00 kHz with tetramethylsilane as a reference. The samples were run in a 10-mm tube with deuterochloroform (30% w/v) as an internal lock and solvent for I–IV and deuterated dimethyl sulfoxide (30% w/v) as an internal lock and solvent for V. The spectrometer settings were: spectral width, 4 kHz; pulse width, 12 μsec (60°); and data points, 4 K. Relaxation time (T_1) measurements of all compounds were carried out in undegassed solutions and were calculated automatically by the computer by least-squares analysis (13) of the plot of ln $(I - I_t)$ *versus* T.

Azapetine phosphate and the hydrochlorides of tolazoline, dibenamine, phenoxybenzamine, and phentolamine, were converted into the corresponding free bases prior to the determination of their ^{13}C-NMR spectra.

RESULTS AND DISCUSSION

Tolazoline (I)—The chemical shifts and relaxation times of various I carbon resonances are recorded in Table I. The SFORD spectrum of I showed two singlets, two doublets, and two triplets. The two singlets farthest downfield having longer relaxation time are due to C-2 and C-7. Since the methyl substituent in the benzene nucleus causes an 8.9-ppm downfield shift to the *ipso*-carbon (11), the singlets at 136.1 and 166.5 ppm are assigned to C-7 and C-2, respectively.

The C-10 chemical shift could be differentiated from the C-8 and C-9 chemical shifts by its shorter relaxation time (T_1). In I, C-10 is *para* to the methylene substituent of the benzene nucleus. It should have a shorter T_1 value compared to C-8 and C-9 due to the anisotropic motion along the preferred long molecular axis, *i.e.*, from C-6 to C-10, where C-8 and C-9 show more motion, resulting in longer T_1 values due to less favorable dipole–dipole interaction (11). Also, methyl substituents in the benzene ring produce 0.1- and 2.9-ppm upfield shifts to *meta*- and *para*-carbons, respectively, and a 0.7-ppm downfield shift to the *ortho*-carbon (11). On this basis and the percent integration of signals, the doublet centered at 126.7 ppm is assigned to C-10 and the doublet at 128.5 ppm is assigned to both C-8 and C-9. These assignments compare well with their calculated values.

Table I—^{13}C-Chemical Shifts of Tolazoline

Assignment[a]	Multiplicity[b]	Relaxation Time[c]	Chemical Shift[d]
C-2	s	15.20	166.5
C-7	s	26.10	136.1
C-8, C-9	d	3.21	128.5
C-10	d	1.68	126.7
C-4, C-5	t	1.02	49.5
C-6	t	2.40	35.7

[a] Numbering of the carbons is shown in the structure. [b] Signal multiplicity obtained from SFORD spectrum; s = singlet, d = doublet, t = triplet, and q = quartet. [c] Relaxation time was measured in undegassed solution. [d] Chemical shifts are expressed in parts per million relative to tetramethylsilane.

[1] Jeol FX 60.

Table II—^{13}C-Chemical Shifts of Dibenamine

Assignment[a]	Multiplicity[b]	Relaxation Time[c]	Chemical Shift[d]
C-4	s	7.68	138.9
C-5[e]	d	1.02	128.5
C-6[e]	d	1.03	128.1
C-7	d	0.75	126.9
C-3	t	0.60	58.3
C-2	t	0.60	55.1
C-1	t	0.98	41.6

[a–d] See Table I. [e] May be interchanged.

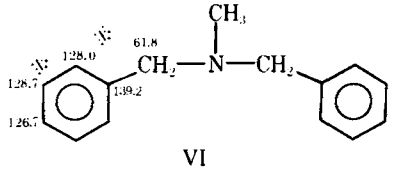
The C-4 and C-5 of the I imidazole ring are directly attached to the nitrogen atom and are in the same environment, so the signals due to these carbons should be observed at the same place. On this basis and the percent integration of the signals, the triplet centered at 35.7 ppm is assigned to C-6 and the triplet at 49.5 ppm is assigned to both C-4 and C-5.

Dibenamine (II)—As is evident from Table II, one singlet and three doublets in the downfield region and three triplets in the upfield region are observed in the ^{13}C-NMR spectrum of II; these signals account for all 16 carbon resonances. The four signals in the downfield region represent the 12 aromatic carbon resonances due to the plane of symmetry. The farthest downfield singlet at 138.9 ppm having a longer relaxation time is attributed to C-4. Considering the effect of a methyl substituent on the benzene nucleus (11) and the relaxation times of C-5, C-6, and C-7, where C-7 should have a shorter T_1 value compared to C-5 and C-6 (11), the doublets centered at 128.5, 128.1, and 126.9 ppm are assigned to C-5, C-6, and C-7, respectively. The assignments of C-5 and C-6 could be interchanged. Furthermore, these assignments compare well with the chemical shifts of the corresponding carbons of I and VI (12).

Previous studies reported that the amino group in aliphatic amines exhibits 20- and 2-ppm downfield shifts to α- and β-carbons, respectively (11). Also, chlorine in alkyl chlorides causes 23- and 2-ppm downfield shifts to α- and β-carbons, respectively (11). Both α- and β-carbons cause an ~9-ppm downfield shift while the γ-carbon produces a 2-ppm upfield shift (11). The chemical shift of the methyl group carbon resonance in toluene is 21.2 ppm (12). The C-3 is directly attached to the nitrogen atom and has two β- and one γ-carbons. Thus, the triplet centered at 58.3 ppm is best assigned to C-3. The C-1 has one α-chlorine, one α- and two γ-carbons, and one β-nitrogen; C-2 has one α- and two β-carbons, one α-nitrogen, and one β-chlorine. Considering these effects on the base peak of ethane (11), the triplets centered at 55.1 and 41.6 ppm are attributed to C-2 and C-1, respectively.

Azapetine (III)—The chemical shifts of the carbon resonances of III obtained from its ^{13}C-NMR spectrum and their relaxation times are recorded in Table III. The structure of III indicates that there is a plane of symmetry that reduces half of the aromatic carbon resonances. The signals at 140.9 and 134.4 ppm, observed as singlets in the SFORD spectrum of III and having longer relaxation times, must be due to C-5 and C-10. A phenyl substituent in the benzene ring produces a 13.1-ppm downfield shift to the *ipso*-carbon and a 1.1-ppm upfield shift to the *ortho*-carbon, and a methyl group causes 8.9- and 0.7-ppm downfield shifts to *ipso*- and *ortho*-carbons, respectively (11). The singlets at 140.9 and 134.3 ppm are assigned to C-10 and C-5, respectively.

The chemical shift of the ethylene carbon resonance is 123.3 ppm (11). The vinylic carbon C-2 has one α- and two γ-carbons and one β-nitrogen while C-1 has one α′- and two γ′-carbons and one β′-nitrogen. The α- and γ′-carbons cause 10.6- and 1.5-ppm downfield shifts, respectively, while γ and α′ produce 1.5- and 7.9-ppm upfield shifts, respectively, in alkenes (11). The β-nitrogen exhibits a 2-ppm downfield shift (11). On this basis, the doublet centered at 136.1 ppm and the triplet centered at 117.7 ppm



VI

Table III—^{13}C-Chemical Shifts of Azapetine

Assignment[a]	Multiplicity[b]	Relaxation Time[c]	Chemical Shift[d]
C-10	s	21.30	140.9
C-2	d	1.80	136.1
C-5	s	15.00	134.3
C-6	d	1.20	129.5
C-7[e]	—	0.87	127.7
C-8[e], C-9[e]	—	1.10	127.4
C-1	t	0.92	117.7
C-3	t	0.75	58.2
C-4	t	0.68	54.5

[a-d] See Table I. [e] See Table II.

are assigned to C-2 and C-1, respectively. The signals at 129.5 and 127.7 ppm are assigned to C-6 and C-7, respectively, and the signal at 127.4 ppm is assigned to both C-8 and C-9 on the basis of chemical shift theory and percent integration of the signals. The assignments of C-7, C-8, and C-9 may be interchanged.

The upfield triplets centered at 58.2 and 54.5 ppm are assigned to C-3 and C-4, respectively, on the basis of chemical shift theory and signal intensity observed in the proton noise-decoupled spectrum of III.

Phenoxybenzamine (IV)—The chemical shifts and relaxation times of the various carbon resonances of IV are recorded in Table IV. Thirteen separate signals in the proton noise-decoupled spectrum account for the 18 carbon resonances of IV. The carbon resonances of C-4 and C-10 could be easily separated from the others due to their longer relaxation times and multiplicities in the SFORD spectrum. Since methoxy and methyl substituents in the benzene nucleus exhibit 31.4- and 8.9-ppm downfield shifts to the *ipso*-carbon (11), respectively, the singlet at 158.7 ppm is assigned to C-10 and the singlet at 140.1 ppm is assigned to C-4. The benzene ring methoxy group causes a 1-ppm downfield shift to the *meta*-carbon and 14.4- and 7.7-ppm upfield shifts to *ortho*- and *para*-carbons, respectively (11). The chemical shift due to the methyl substituent in the benzene ring was already mentioned in the assignment of I carbon resonances. Thus, on the basis of signal intensities, multiplicities in the SFORD spectrum, and effects of methyl and methoxy substituents on the benzene ring, the doublets centered at 129.2, 128.2, 126.9, 120.5, and 114.3 ppm are attributed to C-12, both C-5 and C-6, C-7, C-13, and C-11, respectively. Furthermore, the assignments of C-4, C-5, C-6, and C-7 are in agreement with the corresponding carbon chemical shifts of II.

The farthest upfield quartet centered at 13.1 ppm could be easily assigned to C-14 on the basis of multiplicity and chemical shift theory. The signals at 42.9, 54.5, and 55.6 ppm are assigned to C-1, C-2, and C-3, respectively. These assignments are based on chemical shift theory and comparisons with the corresponding carbon chemical shifts of II. The chemical shift of C-3 is observed upfield compared to C-3 of II because C-3 of IV has two more γ-carbons, which produce about a 2-ppm upfield shift. The C-9 has one α-, one β-, and two γ-carbons, one α-oxygen, and one β-nitrogen while C-8 has two α-, two β-, and one γ-carbons, one α-nitrogen, and one β-oxygen. Considering the chemical shifts caused by carbon, nitrogen, and oxygen atoms (11), the triplet centered at 70.0 ppm is assigned to C-9 and the signal at 52.9 ppm is assigned to C-8.

Phentolamine (V)—The chemical shifts and relaxation times of the V various carbon resonances are shown in Table V. The SFORD spectrum gave five singlets, five doublets, two triplets, and one quartet. The singlets were confirmed by their longer relaxation times. The farthest downfield

Table IV—^{13}C-Chemical Shifts of Phenoxybenzamine

Assignment[a]	Multiplicity[b]	Relaxation Time[c]	Chemical Shift[d]
C-10	s	12.10	158.7
C-4	s	6.80	140.1
C-12	d	0.92	129.2
C-5, C-6	d	0.76	128.2
C-7	d	0.46	126.9
C-13	d	0.48	120.5
C-11	d	1.00	114.3
C-9	t	0.35	70.0
C-3	—	0.43	55.6
C-2	—	0.62	54.5
C-8	—	0.38	52.9
C-1	t	0.57	42.9
C-14	q	0.84	13.1

[a-d] See Table I.

Table V—^{13}C-Chemical Shifts of Phentolamine

Assignment[a]	Multiplicity[b]	Relaxation Time[c]	Chemical Shift[d]
C-2	s	2.60	165.9
C-15	s	2.00	158.3
C-13	s	3.10	149.6
C-7	s	3.70	145.0
C-10	s	2.80	132.2
C-9, C-11, C-17	d	0.34	129.7
C-8, C-12	d	0.31	123.9
C-18	d	0.14	108.2
C-16	d	0.12	107.2
C-14	d	0.18	104.9
C-6	t	0.10	51.1
C-4, C-5	t	0.13	49.1
C-19	q	0.92	20.4

[a-d] See Table I.

singlet at 165.9 ppm is assigned to C-2 by comparing the chemical shift of C-2 in I. Considering the chemical shifts caused by amino, hydroxy, and methyl substituents on the benzene ring (11), the singlets at 158.3, 149.6, 145.0, and 132.2 ppm are assigned to C-15, C-13, C-7, and C-10, respectively. The doublet centered at 129.7 ppm is assigned to C-9, C-11, and C-17, and the doublet at 123.9 ppm is assigned to C-8 and C-12. The remaining doublets at 108.2, 107.2, and 104.9 ppm are assigned to C-18, C-16, and C-14, respectively. The assignments are made on the basis of chemical shift theory (11) and signal intensity.

The quartet centered at 20.4 ppm is attributed to C-19, which agrees well with the toluene methyl carbon chemical shift (12). The triplet centered at 49.1 ppm is assigned to C-4 and C-5 on the basis of signal intensity and chemical shift theory. Furthermore, this assignment is supported by the corresponding carbon chemical shifts of I. The remaining triplet centered at 51.1 ppm is assigned to C-6.

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ACKNOWLEDGMENTS

Supported in part by a research grant from the American Parkinson Disease Association, New York, N.Y., and National Science Foundation Grant CHE 76-05678 for the partial purchase of the ^{13}C-NMR spectrometer.

The authors thank Dr. R. G. Severson and Dr. S. J. Brumleve for their advice and encouragement. Grateful acknowledgment is made to Smith Kline and French Laboratories for the generous gift of tolazoline, dibenamine, and phenoxybenzamine hydrochlorides; Hoffmann-La Roche for azepetine phosphate; Ciba-Geigy Corp. for Regitine (phentolamine) hydrochloride; and the Northwest Area Foundation, St. Paul, Minn., for providing a Hill Professorship to S. S. Parmar.