^{15}N and ^{13}C Nuclear Magnetic Resonance Spectra of Diazo and Diazonium Compounds[1a]

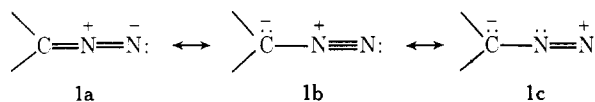**Rudolf O. Duthaler, Hans G. Förster,[1b] and John D. Roberts***

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Abstract: Nitrogen-15 and carbon-13 nuclear magnetic resonance (NMR) spectra have been taken of a number of diazo, benzenediazonium, and azido compounds. The ^{13}C chemical shifts of C1 of these substances are at rather high fields (23–112 ppm for diazo compounds compared to 155–185 ppm for imines, and 102–123 ppm for diazonium salts compared to 148 ppm for nitrobenzene) and do not seem explicable in terms of high charge densities on these carbons. The ^{15}N resonances of diazomethane shift downfield when phenyl groups are substituted on carbon and upfield when the carbon is incorporated as C5 of a cyclopentadiene ring. Substitution of electron-donating and electron-attracting groups in the 4 position of benzenediazonium salts shifts the ^{15}N resonances *downfield* and *upfield*, respectively.

Diazo compounds have stimulated the interest of chemists for many years because of their unusual structures, physical and chemical properties, and their utility in synthesis.[2-4] Recently, several studies have been published of ^{1}H,[5] ^{13}C,[6] ^{14}N,[7] and ^{15}N NMR[8,9] shifts of diazo compounds. The peculiarly large high-field resonance of the C1 carbon of diazo compounds has been given considerable attention, and has been rationalized in terms of counterpart shifts with isoelectric substances such as 1,2-dienes,[10] ketenes,[11] ketenimines,[12] and nitrile oxides.[13] The problem of assigning the nitrogen resonances of diazo and diazonium compounds to the proper nitrogens has been attacked by ^{15}N labeling.[8,9] Calculations of ^{15}N screening constants of this kind of compound have been made, although these have not correlated very well with experiment.[14]

Because the presumed relative importance of ionic resonance structures of the type **1a, 1b,** and **1c** has been invoked to ac-
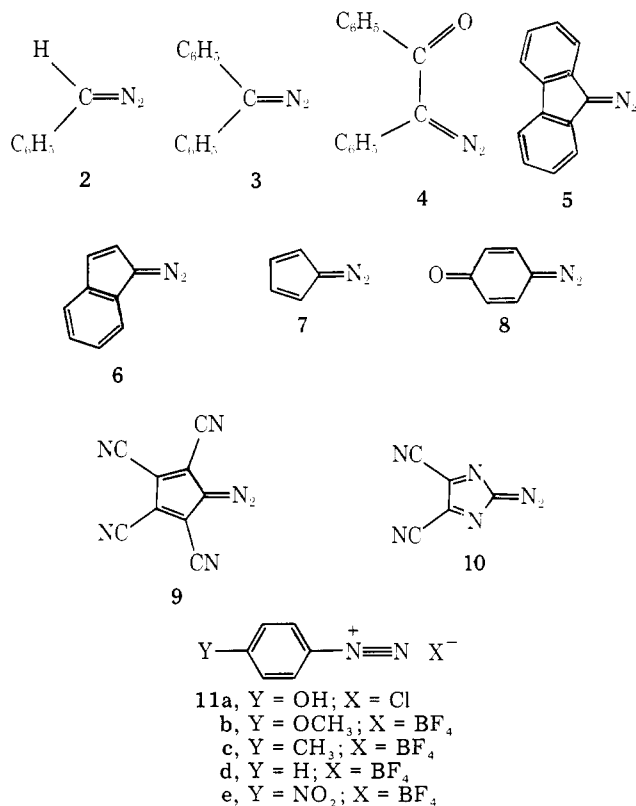

count for changes in the properties of diazo compounds with structure, we have measured the ^{13}C and ^{15}N NMR shifts of a series of diazo and diazonium compounds which would be expected to possess different degrees of ability to stabilize **1b** and/or **1c** relative to **1a.**

Results and Discussion

Table I shows the ^{13}C chemical shifts (referenced to tetramethylsilane) of the C1 (ipso) carbons and ^{15}N chemical shifts (referenced to external nitric acid) of the N1 (central) and N2 (terminal) nitrogens of **2–10** along with those reported for diazomethane (**12**).

The large upfield shifts for the C1 carbons might be taken to reflect substantial contributions of resonance structures of type **1b** or **1c** to the hybrid structure of the ground state and it has been shown that a linear correlation exists between the calculated C1 charge densities and the C1 carbon shifts of 1,2-propadiene, diazomethane, and ketene.[11b] However, matters are perhaps not all that simple, because the ^{13}C shift which corresponds per unit of calculated charge is about 300 ppm, and this is almost double the usual accepted value which suggests that other structural influences must also be operating.[11b]

It is well known that, although the ^{13}C resonances of alkyl carbanion resonances are expected to be far upfield and, indeed, are upfield of the resonances of the carbons of tetramethylsilane, substitution of the anionic carbon with unsatu-

rated groups causes substantial downfield shifts (see Table I). Thus, for the cyclopentadienide ion, the carbon resonances come at about 103 ppm, some 118 ppm downfield from the carbon resonance of methyllithium (which appears to be the best compound so far available for estimating the shift of CH$_3$:$^-$). Because groups attached to the C1 carbon of a diazo compound which stabilize anionic charge should be expected to increase the relative contribution of resonance structures such as **1b** to the hybrid, we might suppose that the C1 shift of the diazo compounds should correlate with the shifts of the corresponding carbons of appropriate carbanions. We show in Figure 1 how these shifts correlate, and the linear fit is, in fact, quite good (slope 0.40, intercept 31.4 ppm, and correlation coefficient 0.994). The point corresponding to CH$_3$:$^-$, representing, as it does, the shift of CH$_3$:$^-$ associated with Li$^+$ in the form of CH$_3$Li, is very likely not to be as far upfield as unassociated CH$_3$:$^-$, and if we omit it from the least-squares analysis, the correlation coefficient becomes 0.995 (slope 0.34 and intercept 36.5 ppm). The predicted shifts of CH$_3$:$^-$ from

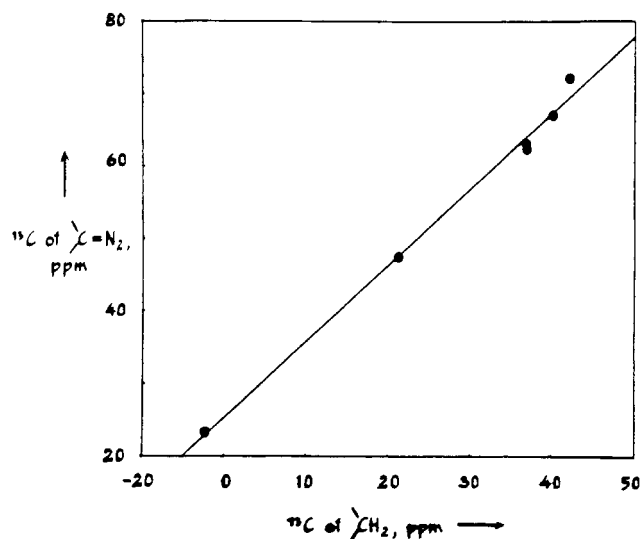**Table I.** ^{13}C and ^{15}N NMR Chemical Shifts of Diazomethyl Carbons and Nitrogens of Diazo Compounds

compd	$\delta^{13}C(1)^a$	solvent^a	$\delta^{13}CH_2{}^b$	$\delta^{13}CH^{-\ c}$	$\delta^{15}N(1)^d$	$\delta^{15}N(2)^d$	solvent
$H_2C=N_2$ (**12**)	23.3^e	CDCl$_3$	−2.3^f	−15.0^g	90^h	−14^h	(C$_2$H$_5$)$_2$O
						−21^i	CDCl$_3$
2	47.2^e	CDCl$_3$	21.3^g	30.7^g	76.8	−62.5	(C$_2$H$_5$)$_2$O
3	62.3^e	CDCl$_3$	37.0^g	78.8^j	70.7^k	−65.5^k	(CH$_2$)$_6$
	62.5	C$_6$H$_6$			70.0	−66.2	(CH$_3$)$_2$CHOH
					69.2	−66.1	(CH$_3$)$_2$SO
4	57.7	CDCl$_3$			92.7	−32.0	(C$_2$H$_5$)$_2$O/(CH$_2$)$_4$O
					95.7	−25.8	CHCl$_3$
5	63.2	CDCl$_3$	36.9^l		89.0^i	−65.0^i	CDCl$_3$
					88.0	−66.4	C$_6$H$_6$
6	67.1	C$_6$H$_6$	40.2^m	91.2^m	95.8	−42.3	CH$_3$(CH$_2$)$_3$CH$_3$
7	72.2	C$_6$H$_6$	41.6^g	103.1^g	106.2	−8.8	CH$_3$(CH$_2$)$_3$CH$_3$
8	79.8	C$_2$H$_5$OH/H$_2$O			117.1	9.6	C$_2$H$_5$OH/H$_2$O
	73.6	CHCl$_3$^{n}					
9	92.6	(CH$_3$)$_2$SO			147.1^o	41.6^o	(CH$_3$)$_2$SO
10	112.2	(CH$_3$)$_2$SO^p			146.0^q	59.4^q	(CH$_3$)$_2$SO

^{a} Downfield from Me$_4$Si in parts per million in the indicated solvent. ^{b} Shift in parts per million downfield from Me$_4$Si of the CH$_2$ carbon in the corresponding hydrocarbon, wherein the diazo nitrogens are replaced by hydrogens. ^{c} Shift in parts per million downfield from Me$_4$Si of CH$^-$ carbon of the anion prepared from the hydrocarbon corresponding to replacement of the diazo nitrogens by hydrogen. Lithium salts in oxacyclopentane, except for **7**, which was the potassium salt in the same solvent. For solvent and concentration influences on these shifts, see ref 18 and 19. ^{d} Upfield from external 1 M H^{15}NO$_3$ in D$_2$O of the diazo nitrogen attached to carbon. Values from the literature have been converted to the nitric acid standard using the relations given in the Experimental Section. ^{e} References 6a and 8. ^{f} Reference 15. ^{g} Reference 16. ^{h} Reference 7. ^{i} Reference 9. ^{j} Reference 19a. ^{k} Shifts of 81.0 and −59.0 ppm have been reported in ref 7 for CH$_2$Cl$_2$ solutions and, what must be erroneous values (possibly through conversion to the (CH$_3$)$_3$N$^+$ standard, because the shift difference is normal), of 126.8 and −9.3 ppm for CDCl$_3$ solutions in ref 8. ^{l} Reference 20. ^{m} Reference 21. ^{n} Reference 17. ^{o} 2,5- and 3,4-cyano nitrogens are not specifically assigned but come at 97.7 and 103.8 ppm. ^{p} The ^{13}C spectrum of **10** showed only two peaks with a 2:3 intensity ratio. We assume that the C1 chemical shift is the same as those of the ring carbons. ^{q} Ring nitrogens and 5,6-cyano nitrogens at 82.7 and 108.0 ppm, respectively.
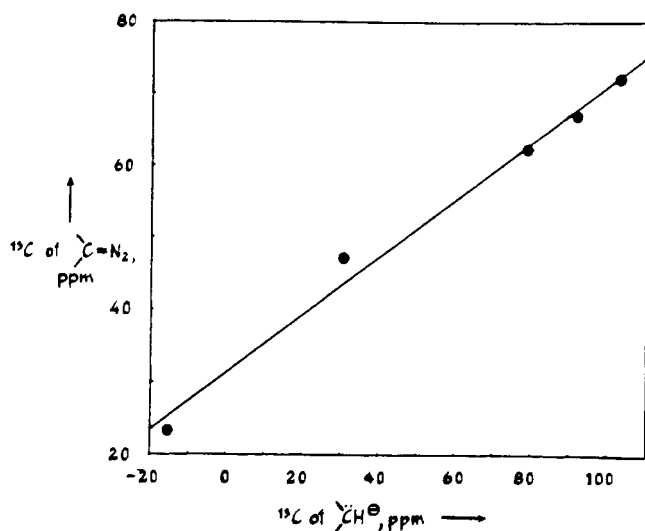
Figure 1. Plot of the ^{13}C shifts of diazomethyl carbons against the ^{13}C shifts of the anions of the hydrocarbons corresponding to replacement of the >C=N$_2$ group by >CH$_2$.

Figure 2. Plot of the ^{13}C shifts of diazomethyl carbons against the ^{13}C shifts of the CH$_2$ carbon formed by replacement of the >C=N$_2$ group by >CH$_2$.

this correlation is the quite reasonable value of −39 ppm. These results would appear to validate our premise that the C1 shifts of diazo compounds should reflect the relative importance of the contribution of resonance structures such as **1b** to the hybrid structure. However, and perhaps surprisingly, an even better correlation is shown in Figure 2, where we have plotted the shifts of the C1 carbons of the diazo compounds against the -CH$_2$- shifts of the corresponding hydrocarbons where the >C=N$_2$ group is replaced by >CH$_2$. The correlation coefficient is 0.997, slope 1.05, and intercept 25.3 ppm. Taken by itself, this correlation suggests that the differences in π-electron density on the sp^2-hybridized C1 carbons of the diazo compounds cannot be any more important to the differences in their respective chemical shifts than the differences in σ-electron

density on the corresponding sp^3-hybridized, *saturated* >CH$_2$ carbons of the hydrocarbons. Although the reasoning may seem to have some aspects of the question as to whether the chicken or the egg came first, it appears to call into question as to just how to best interpret the ^{13}C shifts of the carbanions themselves.

An important problem in connection with the ^{15}N NMR spectra of diazo and diazonium salts that we mentioned earlier is that of telling which of the two ^{15}N resonances should be assigned to the central nitrogen and which to the terminal nitrogen. This has been solved for ethyl diazoacetate and diazocamphor by using ^{15}N labeling[8,9] and, because of its importance, applied here also to diazocyclopentadiene (**7**) through the use of N1- and N3-labeled 4-methylbenzenesul-

fonyl azide which gave **7** labeled with ^{15}N predominantly (but apparently not exclusively) at N1, the central nitrogen (see Figure 4 and later discussion). Similarly, the aryldiazonium nitrogen resonances were assigned with the aid of the spectrum of 4-methylbenzenediazonium ion specifically labeled at N2.

The ^{15}N chemical shifts of the central nitrogens (N1) of the diazo compounds range from 70 ppm for **3** to +147 ppm for **9**. The range of shifts for the terminal nitrogens (N2) is −66 ppm for **3** to +59 ppm for **10**. Benzenediazonium fluoroborate (**11d**) solubilized in chloroform by 18-crown-6 (1,4,8,12,16-hexaoxacyclooctadecane) has its N1 resonance at 150 ppm and N2 resonance at 57 ppm. It is clear that insofar as the nitrogen shifts are concerned, **9** and **10** can be well formulated as dipolar ions with a full $-N^+\equiv N$ group (**13** and **14**).


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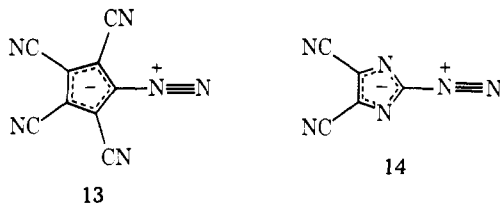
We should note, however, that there is one aspect of the sequence of shifts for compounds **2–7** which does not accord with the diazo nitrogen shifts being determined by the relative importance of resonance forms **1a**, **1b**, and/or **1c**. This is the trend of substantial *downfield* shifts of 13–20 and 41–45 ppm at N1 and N2, respectively, produced by substitution of one or two phenyl groups (**2** and **3**, respectively) on diazomethane. Phenyl groups are well known for their ability to delocalize anionic charge and therefore should be expected to increase the diazonium character of the diazo nitrogen, **15**. The


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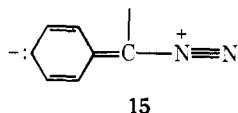
shielding differences of about 3 ppm of the ^{13}C resonances of the 4-carbons of phenyldiazomethane have been explained by such delocalization.[8] The observed ^{15}N shifts of diphenyldiazomethane, however, are *opposite* to the expected direction from the nitrogens of diazomethane.

An important difference between the mono- and diphenyldiazomethanes and the other diazo compounds studied is in their electronic spectra. The series **5–10** shades from red or orange to colorless while **2** and **3** range from red to permanganate purple. This fact suggests that phenyl groups substituted on the carbon of diazomethane do something different to the electronic structure than just enhancing the contributions of resonance structures of the type **1b**. One possibility suggested by the Walch and Goddard structure for diazomethane[3] is a significant contribution of resonance structures such as **16**,

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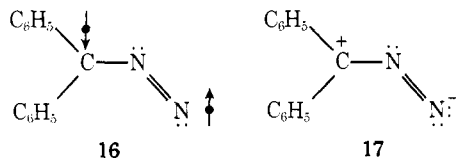
which would surely entail concomitant delocalization involving the π-electron systems of the phenyl substituents. An alternative would be contributions of structures suggested by **17**, which would also be expected to involve the phenyl substituents. Resonance structures related to either **16** or **17** could substantially change the second-order paramagnetic contribution to the chemical shifts of the nitrogens.[22] That phenyl substitution produces both downfield shifts of the nitrogens and a larger λ_{max} for electronic excitation is as expected for

enhanced contribution of the second-order paramagnetic effect operating directly at each nitrogen. Resonance involving forms corresponding to either **16** or **17** are not expected to be favored for diazo compounds **7–10**, the carbon parts of which are well known to be unfavorable as radicals or cations. Thus, it is not surprising on the basis of our hypothesis that **7–10** have both upfield nitrogen shifts and larger energies for electronic excitation. Azibenzil (**4**) offers an interesting competition between a phenyl and an acyl group for producing contributions of resonance structures related to **1b** and **16** (or **17**). The nitrogen shifts are substantially upfield of those for **2**, but not greatly different from those for **12**, which indicates more or less equal influences on the electron structure. The contribution of the diazonium-type structures is apparently substantially increased by changing solvent from diethyl ether/oxacyclopentane to chloroform which, through hydrogen bonding, **18**, causes up-


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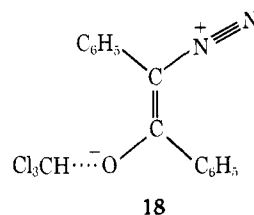
field shifts of the nitrogens. ^{1}H NMR data which support substantial hydrogen bonding have been reported for other diazo ketones and diazosulfones.[23] The pattern of nitrogen shifts reported for diazocarbonyl compounds is in accord with our interpretation, as can be seen from the respective N1, N2 shifts of diazoacetophenone (104.6, −1 ppm),[8] 3-diazocamphor (106, −28 ppm),[9] ethyl diazoacetate (105.2, −11.1 ppm),[8] and diethyl diazomalonate (116.9, −2.4 ppm).[8] The reported shifts[8] were converted to the nitric acid scale by the relation $\delta = \delta_{rep} + 332.8 - 56.7$.

Similar conflicts between electronic structural influences are expected and observed in the pattern of nitrogen shifts for **5** and **6** where the benzene rings should tend to favor **16** or **17**, while the cyclopentadiene rings should increase contributions of **1b**. The influence of the two benzene rings in **5** makes for downfield shifts, while **6** is intermediate between **5** and **7** because the cyclopentadiene influence is proportionately larger than for **5**.

A very interesting aspect of contributions of resonance structures related to **16** or **17** to diazo compounds such as **2** and **3** is the possibility that such contributions should tend to make the ∠C-N-N less than 180°. It would be strong confirmation of the postulated contributions if, in fact, the C-N-N bond angles of **2** and **3** were less than 180°.

The shifts of the 4-substituted benzenediazonium salts listed in Table II seem to be less complicated. It is well known from many chemical and physical techniques (for example, infrared spectra[26] and ^{13}C NMR shifts[17]) that the diazonium group is strongly electron accepting. Electron release by substituents at the 4 position of benzenediazonium salts therefore leads to larger contribution of resonance structures such as **19b**, which

19a

19b
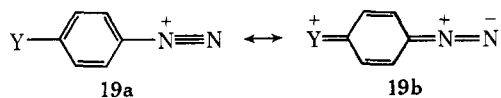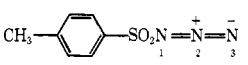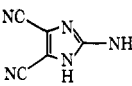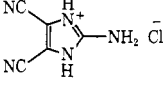
reaches the ultimate with Y = O^-, which is conventionally written with bond structure **8**. The effect of electron-donating groups is to increase the diazo character, **1**, and produce downfield shifts of the nitrogens. The relatively small differences in shift between N2 of benzenediazonium ion and 4-nitrobenzenediazonium ion suggests that there also may be some sort of an electron-donating effect of the nitro group which counters its usual, expected electron-attracting influ-

Table II. ^{13}C and ^{15}N NMR Chemical Shifts of 4-Substituted Benzenediazonium Salts

4 substituent		$\delta^{13}C(1)^a$	$\delta^{15}N(1)^b$	$\delta^{15}N(2)^b$
$-O^-$	**8**	79.8^c	117.1^c	9.6^c
$-OH$	**11a**	102.1	146.8	50.8
$-OCH_3$	**11b**	105.0^d	148.5	53.2
		103.1^e		
$-CH_3$	**11c**	113.3	149.4	56.9
		109.1^d		
		111.5^e		
$-H$	**11d**	115.8^d	150.2	57.2
		115.2^e	144.5^f	65^f
$-NO_2$	**11e**	123.0^d	152.2	57.1
		121.8^e		

^{a} Downfield from Me$_4$Si; the solvent was CHCl$_3$ containing 18-crown-6, if not noted otherwise. ^{b} Upfield from external 1 M H^{15}NO$_3$, the solvent was CHCl$_3$ containing 18-crown-6; the counterion was BF$_4^-$ except for **8** (no counterion) and **11a**, for which the counterion was Cl$^-$. ^{c} In ethanol/water solution. ^{d} In liquid sulfur dioxide, ref 24. ^{e} In acetonitrile, ref 17. ^{f} In methanol/acetone, ref 25.

Figure 3. ^{15}N NMR absorption peak of N1 of 4-methylbenzenesulfonyl azide in cyclohexane; the peak is at 237.2 ppm, with a line width at half-height of about 31 Hz.

Table III. ^{15}N NMR Chemical Shifts of Some Related Compounds

compd	solvent	$\delta^{15}N^a$	assign-ment^b
CH$_3$—⟨⟩—SO$_2$N$\overset{+}{=}$N$\overset{-}{=}$N (1, 2, 3)	(CH$_2$)$_6$	237.2^c	N(1)
		141.9^c	N(2)
		134.0^c	N(3)
	CH$_3$OH	142.4	N(2)
		133.2	N(3)
NC, N, NC, H, NH$_2$ (ring)	(CH$_3$)$_2$SO/ CH$_3$OH	105.6	CN
		195.6	ring N^d
		320.4	NH$_2$
NC, H, N$^+$, NC, H, NH$_2$ Cl$^-$ (ring)	(CH$_3$)$_2$SO/ H$_2$O/HCl	103.2	CN
		199.1	ring N
		313.1	NH$_2$
⟨⟩—^{15}N$=$N—$\overset{+}{P}$(C$_6$H$_5$)$_3$ (1, 2)	CH$_2$Cl$_2$/ CHCl$_3$	-50.3	N(1)^e

^{a} Upfield from external 1 M H^{15}NO$_3$ in D$_2$O. ^{b} Assignments based on ^{15}N labeling or on signal intensities and by comparison with model compounds. ^{c} Reference 27 reports ^{14}N shifts and assignments which correspond to 304, 152, and 209 ppm. We cannot reconcile these shifts with ours. ^{d} Averaged by hydrogen exchange. ^{e} $J_{N-P} = 18.5$ Hz.

(a) (b)

-8.9 106.1 -9.2 106.0

Figure 4. ^{15}N NMR resonance lines of diazocyclopentadiene (**7**) in pentane solution: (a) formally expected to be labeled at N1 with ^{15}N to the extent of 48.6%; (b) 5%. The downfield peak corresponds to the N2 resonance in the spectrum at the natural-abundance level with enhanced intensity.

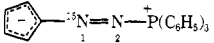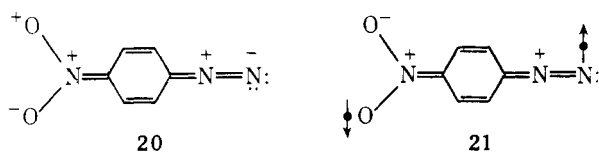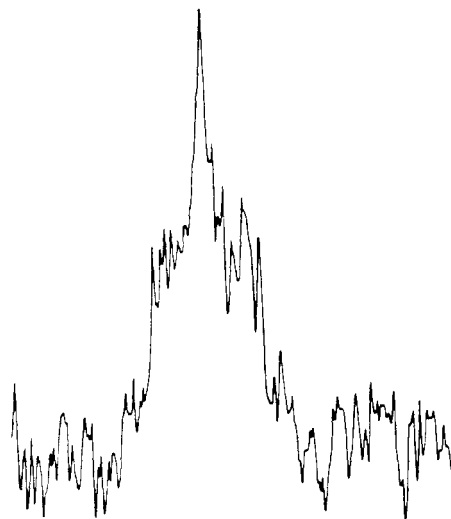ence. This could be the result of small contributions of resonance structures such as **20** (which violate the adjacent charge

20 **21**
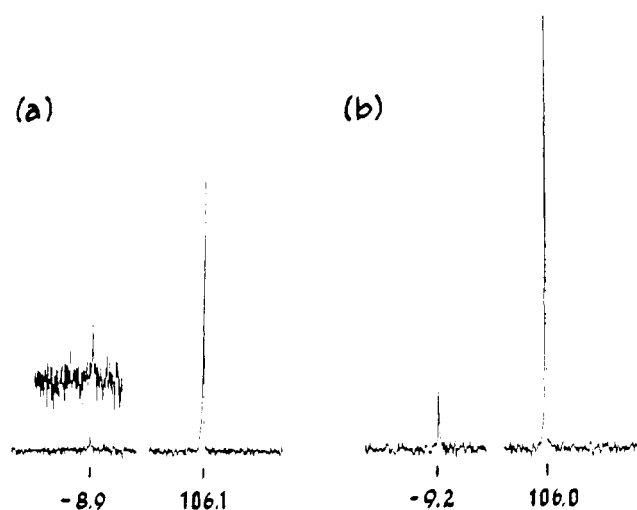
rule), or **21**, which reflects the ability of the nitro group to stabilize a radical center.

The ^{15}N NMR shifts obtained for some precursors and related compounds are reported in Table III.

The ^{14}N chemical shifts of 4-methylbenzenesulfonyl azide have been reported,[27] but the ^{15}N shifts we find (Table III) are very different. Our shift assignments were verified with a sample of the azide which was a 1:1 mixture of isotope position isomers labeled to the extent of 97.2% at N1 and at N3, and an additional sample labeled to the extent of 5% only at N3. Interestingly, the high-field N1 resonance of the neat azide is broader than the low-field N2 and N3 resonances. This 6-Hz resonance further broadens to 14 Hz when the neat azide is

mixed with an approximately equal volume of benzene, and to about 30 Hz when this mixture is further diluted with 11 volumes of cyclohexane (Figure 3). The line broadening can be ascribed to changes in the rate of quadrupole relaxation of ^{14}N2 which, in turn, changes the contribution of the ^{15}N1-^{14}N2 spin–spin coupling to the line width. Similar effects have been reported on the ^{13}C resonance of C1 in diazo compounds[6b,8] and also seem to occur sometimes with the N2 absorptions of diazo compounds.

The ^{15}N spectra of diazocyclopentadiene prepared from the two differently ^{15}N-labeled samples of 4-methylbenzenesulfonyl azide are shown in Figure 4. According to the assumed mechanism for this nitrogen-transfer reaction[37,40] only the N1 nitrogen resonances should have intensities greater than expected for natural abundance. However, the N$_2$ resonances were rather more strongly intense than expected and the experimental intensity ratios for N1/N2 were 19:1 and 6:1, in contrast to expected ratios of 133:1 and 13:1, respectively. Because no label at N2 could be observed for the starting azide, and the ratios of the labels at the various nitrogens in the products did not change after several weeks, we can only

conclude that 5–7% of the label became incorporated at N2 in the synthetic procedure, contrary to what has been reported earlier.[5a] The mechanism by which this isotope-position isomerization takes place is now known[5c] and will be the subject of a later publication.

Experimental Section

Natural-abundance ^{15}N NMR spectra were taken at 18.25 MHz with a Bruker WH-180 spectrometer using 16–20 mL of 0.6–2 M solutions in 25-mm sample tubes. Lock and reference signals were provided by a concentric 5-mm tube containing 1 M 95% $H^{15}NO_3$ in D_2O solution. Some of the samples decomposed, slowly evolving nitrogen gas. When this occurred, 2 mL of benzene-d_6 was added to serve as the source of the lock signal, and after 500–1000 scans, the concentric tube was removed to prevent pressure buildup.

The benzenediazonium salts were solubilized in chloroform by addition of 1.2–1.8 equiv of 18-crown-6.[28]

Chemical shifts are reported here with reference to external 1 M $H^{15}NO_3$, with positive numbers being upfield. The bulk-susceptibility corrections for this reference are about −0.11 ppm for chloroform and +0.76 ppm for ether;[29] however, the reported shifts are uncorrected. This reference is 6.2 ppm upfield from neat nitromethane and downfield by 298.7, 332.8, and 355.0 ppm from 2 M urea in water, 2 M tetramethylammonium chloride in water, and the ammonium ion in 2 M ammonium nitrate in water, respectively. The spectra of the diazo and diazonium compounds were taken without proton decoupling and accumulation of 1000–4000 transients from 20-μs pulses (25° flip angle) with 10–30 s repetition rates gave reasonable signal-to-noise ratios. The ^{13}C NMR spectra were taken at 25.2 MHz with a Varian XL-100 spectrometer and the reported shifts are downfield from internal tetramethylsilane (Me_4Si) or corrected from other internal standards to the Me_4Si scale by the relations $\delta(Me_4Si)$ = $\delta(CHCl_3)$ + 77.1 ppm ≈ $\delta(CH_3OH)$ + 49.9 ppm = $\delta(Me_2SO)$ + 40.4 ppm.

Benzenediazonium and 4-methyl-, 4-nitro-, and 4-methoxy-substituted benzenediazonium fluoroborates were prepared by diazotization of the corresponding benzenamines.[30] 4-Methylbenzenamine with the aid of 5% ^{15}N-enriched sodium nitrite gave 4-methylbenzenediazonium fluoroborate specifically labeled at the terminal nitrogen. 4-Hydroxybenzenediazonium chloride was made by diazotization of 4-aminobenzenol with 3-methylbutyl nitrite and hydrogen chloride in ethanol.[31] Part of the product was converted to diazoquinone (8) with silver nitrate.[32] The ethanol solution was filtered through alumina (grade 1, Woelm neutral), then concentrated at 0°C under reduced pressure to give an approximately 0.6 M solution which was used without further purification.

Azibenzil (4) was obtained by oxidation of benzil monohydrazone with mercuric oxide.[33] Treatment of 4 with sodium hydroxide, ether, methanol, and water gave an ethereal solution of phenyldiazomethane (2).[34] The solution from a 50-mmol run was concentrated to 20 mL by passing a stream of dry nitrogen over it, and the ^{15}N spectrum taken after addition of 2 mL of benzene-d_6 to provide the field-frequency lock signal. Diphenyldiazomethane (3)[35] and diazofluorene (5)[36] were prepared by oxidation of the corresponding hydrazones with yellow mercuric oxide.

4-Methylbenzenesulfonyl azide was prepared from 4-methylbenzenesulfonyl chloride and sodium azide in ethanol.[37] The product contained about 20% of ethyl 4-methylbenzenesulfonate. *N,N*-Dimethylmethanamide as solvent gave a purer product but lower yields. The labeled sulfonyl azide was prepared by adding a solution of 0.50 g of potassium azide enriched to 97.2% [15]N at one terminal nitrogen in 1.5 mL of water to 1.18 g of freshly sublimed 4-methylbenzenesulfonyl chloride dissolved in 7 mL of *N,N*-dimethylmethanamide. The mixture was stirred at room temperature for 1 h, diluted with 100 mL of water, and extracted with a pentane/ether mixture. The extract was washed with water and dried over sodium sulfate and the solvent was removed under reduced pressure.

The sulfonyl azide was also prepared in 94% yield, labeled to the extent of 5% with ^{15}N at the terminal nitrogen, by diazotization of 4-methylbenzenesulfonyl hydrazide with sodium nitrite 5% enriched in ^{15}N by the procedure of Curtius and Kraemer.[38]

Diazoindene was obtained mixed with indene and 4-methylbenzenesulfonyl azide from the reaction of indene and the sulfonyl azide at room temperature in diethylamine solution.[39,40] Most of the indene could be removed by fractional distillation at about 40 °C and a

pressure of 0.1 mm (bath temperature at 50–70 °C). Two distillations of the residue at 60–90 °C and 0.005 mm in a Kugelrohr Apparatur gave a 17% yield of diazoindene containing only a small amount of the 4-methylbenzenesulfonyl azide. Increasing the reaction time from 3 days to 4 weeks did not improve the yield, probably because of side reactions. Substitution of diisopropylamine for diethylamine gave a much slower reaction.

Diazocyclopentadiene was prepared by the method of Weil and Cais.[40] The ^{15}N-enriched material, 5 or 48.6% at N1, was obtained with the labeled 4-methylbenzenesulfonyl azide described above. For the ^{15}N NMR spectrum, the final pentane extract of the product was concentrated to 15 mL under a 15-cm Vigreux column. For the ^{13}C spectrum, the pentane was removed completely and the diazo compound codistilled with benzene at a pressure of 20 mm.

Treatment of an ethereal solution of 7 labeled 5% with ^{15}N at N1 with excess triphenylphosphine caused separation of an oil which solidified at 0 °C and was recrystallized from a dichloromethane/cyclohexane solution.[40] The yield of the triphenylphosphonium complex, **22**, was 77% based on the sulfonyl azide. The complex decomposes in chloroform containing traces of acids.

22
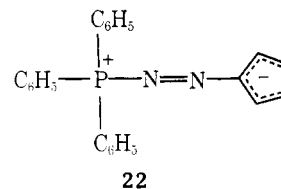
2-Diazo-3,4-dicyanoimidazole (**10**) precipitated on diazotization of 2-amino-3,4-dicyanoimidazole with sodium nitrite in hydrochloric acid.[41] The product was removed by filtration, pressed as dry as possible, then dissolved in dimethyl-d_6 sulfoxide for the ^{15}N and ^{13}C NMR spectra.

References and Notes

(1) (a) Supported by the National Science Foundation, and by the Public Health Service, Research Grant 11072, from the Division of General Medical Sciences; (b) Deutscher Akademischer Austauschdienst NATO Fellow, 1976–1977.
(2) For reviews: (a) H. Zollinger, "Azo and Diazo Chemistry", Interscience, New York, N.Y., 1961; (b) C. G. Overberger, J.-P. Anselme, and J. G. Lombardino, "Organic Compounds with Nitrogen-Nitrogen Bonds", Ronald Press, New York, N.Y., 1966; (c) D. M. Regitz, "Diazoalkane", Georg Thieme Verlag, Stuttgart, 1977.
(3) S. P. Walch and W. A. Goddard, III, *J. Am. Chem. Soc.*, **97**, 5319 (1975).
(4) G. Leroy and M. Sana, *Theor. Chim. Acta*, **33**, 329 (1974).
(5) (a) J. P. Jacobsen, K. Schaumburg, and J. T. Nielsen, *J. Magn. Reson.*, **13**, 372 (1974); (b) A. Ledwith and E. C. Friedrich, *J. Chem. Soc.*, 504 (1964); (c) unpublished experiments by Carla Casewit.
(6) (a) J. Firl, W. Runge, and W. Hartmann, *Angew. Chem.*, **86**, 274 (1974); *Angew. Chem., Int. Ed. Engl.*, **13**, 270 (1974); (b) W. Runge and J. Firl, *Z. Naturforsch. B.*, **31**, 1515 (1976). See also (c) R. Grüning, P. Krommes, and J. Lorberth, *J. Organomet. Chem.*, **127**, 167 (1977).
(7) J. Mason and J. G. Vinter, *J. Chem. Soc., Dalton Trans.*, 2522 (1975).
(8) T. A. Albright and W. J. Freeman, *Org. Magn. Reson.*, **9**, 75 (1977).
(9) D. G. Morris and A. M. Murray, *J. Chem. Soc., Perkin Trans. 2*, 1579 (1976).
(10) (a) R. Steur, J. P. C. M. van Dongen, M. J. A. de Bie, W. Drenth, J. W. de Haan, and L. J. M. van de Ven, *Tetrahedron Lett.*, 3307 (1971); (b) J. K. Crandall and S. A. Sojka, *J. Am. Chem. Soc.*, **94**, 5084 (1972); (c) W. Runge and J. Firl, *Ber. Bunsenges. Phys. Chem.*, **79**, 907 (1975); (d) *ibid.*, **79**, 913 (1975); (e) W. Runge and W. Kosbahn, *ibid.*, **80**, 1330 (1976).
(11) (a) J. Firl and W. Runge, *Angew. Chem.*, **85**, 671 (1973), *Angew. Chem., Int. Ed. Engl.*, **12**, 668 (1973); (b) J. Firl and W. Runge, *Z. Naturforsch. B*, **29**, 393 (1974); (c) G. A. Olah and P. W. Westerman, *J. Am. Chem. Soc.*, **95**, 3706 (1973).
(12) J. Firl, W. Runge, W. Hartmann, and H.-P. Utikal, *Chem. Lett.*, 51 (1975).
(13) M. Christl, J. P. Warren, B. L. Hawkins, and J. D. Roberts, *J. Am. Chem. Soc.*, **95**, 4392 (1973).
(14) K. A. K. Ebraheem and G. A. Webb, *Org. Magn. Reson.*, **9**, 248 (1977).
(15) E. Breitmaier and W. Voelter, "^{13}C-NMR Spectroscopy", Verlag Chemie, Weinheim/Bergstr., Germany, 1974.
(16) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972.
(17) Y. A. Ustynyuk, O. A. Subbotin, L. M. Buchneva, V. N. Gruzdneva, and L. A. Kazitsyna, *Dokl. Akad. Nauk SSSR*, **227**, 101 (1976).
(18) L. D. McKeever in "Ions and Ion Pairs in Organic Chemistry", Vol. 1, M.

Szwarc, Ed., Wiley, New York, N.Y., 1972.
(19) (a) K. Takahashi, Y. Kondo, R. Asami, and Y. Inoue, *Org. Magn. Reson.*, **6**, 580 (1974); (b) D. H. O'Brien, A. J. Hart, and C. R. Russell, *J. Am. Chem. Soc.*, **97**, 4410 (1975); (c) D. H. O'Brien, C. R. Russell, and A. J. Hart, *ibid.*, **98**, 7427 (1976).
(20) J. B. Stothers, C. T. Tan, and N. K. Wilson, *Org. Magn. Reson.*, **9**, 408 (1977).
(21) G. A. Taylor and P. E. Rakita, *Org. Magn. Reson.*, **6**, 644 (1974).
(22) M. Witanowski and G. A. Webb, "Nitrogen NMR", Plenum Press, New York, N.Y., 1973.
(23) J. B. F. N. Engberts and G. Zuidema, *Recl. Trav. Chim. Pays-Bas*, **89**, 741 (1970).
(24) G. A. Olah and J. L. Grant, *J. Am. Chem. Soc.*, **97**, 1546 (1975).
(25) N. N. Bubnov, K. A. Bilevitch, L. A. Poljakova, and O. Y. Okhlobystin, *J. Chem. Soc., Chem. Commun.*, 1058 (1972); see also N. A. Porter and J. G. Green, *Tetrahedron Lett.*, 2667 (1975).
(26) N. C. Cutress, T. B. Grindley, A. R. Katritzky, M. V. Sinnott, and R. D. Topsom, *J. Chem. Soc., Perkin Trans. 2*, 2255 (1972).
(27) W. Beck, W. Becker, K. F. Chew, W. Derbyshire, N. Logan, D. M. Revitt, and D. B. Sowerby, *J. Chem. Soc., Dalton Trans.*, 245 (1972).
(28) G. W. Gokel and D. J. Cram, *J. Chem. Soc., Chem. Commun.*, 481 (1973);

S. H. Korzeniowski, R. J. Petcavich, M. M. Coleman, and G. W. Gokel, *Tetrahedron Lett.*, 2647 (1977).
(29) J. Homer, *Appl. Spectrosc. Rev.*, **9**, 1 (1975).
(30) (a) D. Y. Curtin and J. A. Ursprung, *J. Org. Chem.*, **21**, 1221 (1956); (b) E. B. Starkey, "Organic Syntheses", Collect. Vol. II, Wiley, New York, N.Y., 1943, p 225.
(31) L. C. Anderson and M. J. Roedel, *J. Am. Chem. Soc.*, **67**, 955 (1945).
(32) M. Puza and D. Doetschman, *Synthesis*, 481 (1971).
(33) C. D. Nenitzescu and E. Solomonica in ref 30(b), p 496.
(34) P. Yates and B. L. Shapiro, *J. Org. Chem.*, **23**, 759 (1958).
(35) L. I. Smith and K. L. Howard, "Organic Syntheses", Collect Vol. III, Wiley, New York, N.Y., 1955, p 351.
(36) H. Staudinger and O. Kupfer, *Ber.*, **44**, 2197 (1911).
(37) W. von E. Doering and C. H. DePuy, *J. Am. Chem. Soc.*, **75**, 5955 (1953).
(38) T. Curtius and G. Kraemer, *J. Prakt. Chem.*, **125**, 323 (1930).
(39) D. Rewicki and C. Tuchscherer, *Angew. Chem.*, **84**, 31 (1972), *Angew. Chem., Int. Ed. Engl.*, **11**, 44 (1972).
(40) T. Weil and M. Cais, *J. Org. Chem.*, **28**, 2472 (1963).
(41) W. A. Sheppard and O. W. Webster, *J. Am. Chem. Soc.*, **95**, 2695 (1973).

Studies of pH and Anion Complexation Effects on L-Arginine by Natural Abundance ^{15}N Nuclear Magnetic Resonance Spectroscopy[1]

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Abstract: Natural-abundance nitrogen-15 nuclear magnetic resonance (NMR) spectroscopy has been used to investigate (1) the effect of pH on the ^{15}N chemical shifts of L-arginine and (2) the possible effects on ^{15}N chemical shifts and T_1 values of the complexation of L-arginine with chloride ions, phosphate ions, and adenosine triphosphate (ATP) in aqueous solutions. Guanidine carbonate solutions and protamine sulfate solutions containing some of these ions have also been examined. On protonation of the guanidinium group of L-arginine, the guanidino nitrogens and the –NH– nitrogen show upfield shifts of 15 and 3 ppm, respectively, as a result of change in the second-order paramagnetic effect, while the α-amino nitrogen shows an 8-ppm downfield shift on protonation as a result of decreased shielding. Complexation with equimolar phosphate ions produces a small downfield shift of approximately 0.8 ppm in the chemical shift of the guanidino nitrogens of L-arginine. No significant changes in ^{15}N shifts or T_1 values were observed on complexation with chloride ions or adenosine triphosphate.

Several enzymes have been found to contain arginyl residues which appear to interact with negatively charged phosphate or carboxylate groups of substrates or cofactors.[2] Chemical modification of positively charged arginyl residues (and the resulting loss of charge) has been shown to result in deactivation of the enzymes.[2] Riordan and co-workers have further suggested that enzymes which interact with anionic substances or cofactors will probably contain arginyl residues at the active site.[3] The binding of DNA with histone and protamine is considered to involve electrostatic interactions between the phosphate anions of DNA and the positively charged arginyl and lysyl residues of these nucleoproteins. The guanidinium group of methylguanidine has been shown by x-ray crystallography to complex with a phosphate anion through multiple hydrogen bonds.[4] The complexation of L-arginine with phosphate and chloride ions in aqueous solution has been studied by ^{31}P and ^{35}Cl nuclear magnetic resonance spectroscopy. Katz and co-workers[5] have reported a downfield shift of 0.5 ppm in ^{31}P chemical shift of methyl phosphate on addition of L-arginine in D_2O solution at pH 6.15. Jönsson and co-workers[6] have measured the pH dependence of ^{35}Cl chemical shift in an aqueous mixture of NaCl and L-arginine and observed a distinct change in ^{35}Cl shift on deprotonation of the guanidinium group. The present study was designed (1) to determine a complete ^{15}N chemical shift–pH titration curve

for L-arginine, and (2) to investigate the utility of ^{15}N NMR for detection and characterization of complexations of guanidinium ion (1) and L-arginine (2) with fluoroborate ions, chloride ions, phosphate ions, and ATP in water solutions.

guanidinium ion (1)   arginine (2)
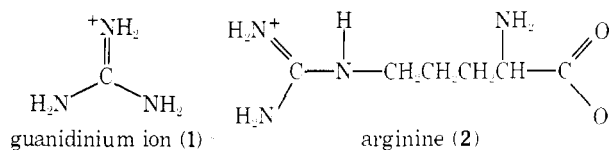
Experimental Section

Natural-abundance ^{15}N NMR spectra were recorded using the pulse Fourier-transform technique with a Bruker WH-180 spectrometer operating at 18.25 MHz. An external 1 M $H^{15}NO_3/H_2O$ capillary reference was used in 25-mm spinning sample tubes. Normal operating conditions for guanidine carbonate and arginine solutions (for spectra other than T_1 measurements) were 55-μs pulses (70° flip angle) with a 15-s pulse delay, and for protamine solutions were 60-μs pulses with a 0.58-s delay. With full proton decoupling, the sample temperatures were about 55° C. Spectra were obtained of 2 M aqueous solutions of guanidine carbonate with internal D_2O field-frequency locks, and the L-arginine spectra were similarly taken of 1 M aqueous solutions. Protamine sulfate spectra were of 0.0315 M aqueous solutions. L-Arginine was purchased from Sigma Chemical Co., and guanidine carbonate from Matheson Coleman and Bell.