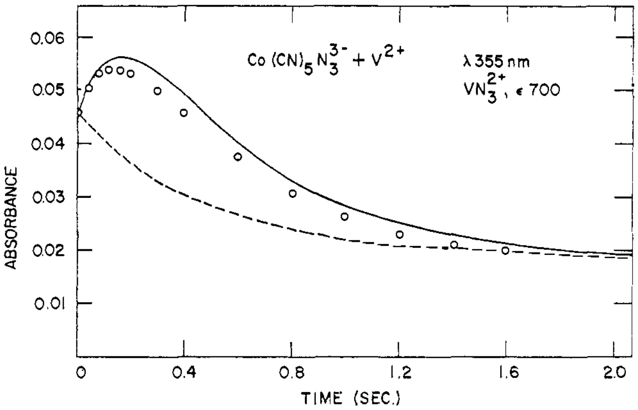

Figure 2. Comparison of the observed absorbance–time values in the reaction of $Co(CN)_5N_3^{3-}$ and V^{2+} with those calculated for the inner-sphere (solid) and outer-sphere (dashed) mechanisms.

position,[2,12] but the V(III) complex $V(H_2O)_5X^{2+}$ as well. Consideration of the various known values of the molar absorptivities and rate constants in the scheme shown in eq 4–6 reproduced the experimental data

$$[Co(CN)_5N_3^{3-}\cdot V^{2+}] \longrightarrow Co(CN)_5^{3-} + VN_3^{2+} \qquad (4)$$

$$Co(CN)_5^{3-} + 5H^+ \longrightarrow Co^{2+} + 5HCN \qquad (5)$$

$$VN_3^{2+} + H^+ \longrightarrow V^{3+} + HN_3 \qquad (6)$$

quite well. The agreement of the observed absorbance–time trace and that calculated for an inner-sphere mechanism is shown in Figure 2. Along with this comparison, the pattern predicted had the reaction followed an outer-sphere mechanism is also shown. The results indicate that VN_3^{2+} is formed in eq 4 at a concentration in great excess over that dictated by the eventual equilibrium in eq 6, thus providing direct "product criterion" evidence for an inner-sphere mechanism is support of the conclusions based on the rate constants cited above.

(12) J. H. Espenson and J. R. Pipal, *Inorg. Chem.*, 7, 1463 (1968).

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Metalation Reactions. I. Formation and Charge Distribution in Conjugated Propargylic Dianions

Sir:

We wish to report the unusually easy formation of dicarbanions by the abstraction of two protons from the same carbon atom in hydrocarbons. These anions have unexpected spectral characteristics indicating increased electron localization on introduction of the second charge in the molecule.

trans-1-Phenylhex-4-en-1-yne (Ia), 1-phenyl-4-methylpent-4-en-1-yne (Ib), *trans*-1,5-diphenylpent-4-en-1-yne (Ic), and 1-phenylpent-4-en-1-yne (Id) were prepared by coupling phenylacetylenemagnesium bromide with the corresponding allyl bromide.[1] 2,7,7-Trimethylhept-1-en-4-yne (Ie) was prepared in a similar manner from *t*-butylacetylenemagnesium bromide and methallyl bromide.

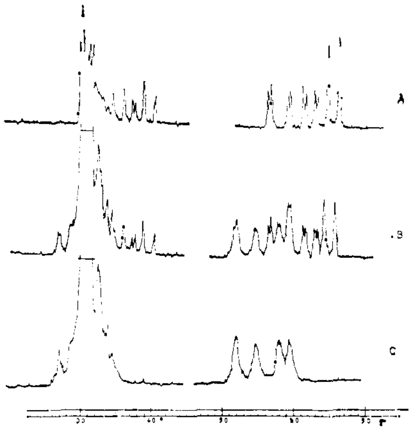
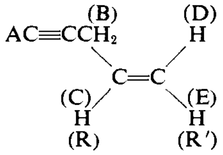


Figure 1. Nmr spectrum of a solution formed by addition at −90° of 100 mg of Id to 1 ml of 3.0 *F* butyllithium in deuterated ether. The spectrum was recorded at 38°: (A) 3 min after the addition of Id (monoanion IId); (B) 30 min after the addition of Id; (C) 120 min after the addition of Id (dianion IIId).

Addition of the compounds Ia–d to an excess of a solution of butyllithium in ether or deuterated ether at −80° produces first a very fast, and then a second, change in their nmr spectra[2] (Figure 1, Table I). The reaction observed consists in consecutive abstractions of one and then a second proton form the propargylic methylene group, giving respectively the mono- (II) and dilithium (III) derivatives.[3] The reaction of Ie is slower but proceeds smoothly to the dianion IIIe. The presence of a phenyl group in these molecules is therefore not a necessary condition for dianion formation.

$AC{\equiv}CCH_2CR{=}CHR'$

Ia, R = H; R′ = CH_3; A = C_6H_5
b, R = CH_3; R′ = H; A = C_6H_5
c, R = H; R′ = C_6H_5; A = C_6H_5
d, R = H; R′ = H; A = C_6H_5
e, R = CH_3; R′ = H; A = $(CH_3)_3C$

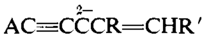


I′

No proton is abstracted from the vinyl group, as shown by integration and the invariance of the coupling constants in this group during the reaction. The nmr spectrum of III is not that of a product formed other than by transmetalation, since protonation yielded the starting material, conjugated enynes, and allenes conjugated to a double bond.[5] Treatment with trimethylsilyl chloride gave the bis(trimethylsilyl) derivatives.[5]


$AC{\equiv}CC\overset{-}{H}CR{=}CHR'$

II


$AC{\equiv}C\overset{2-}{C}CR{=}CHR'$

III

Nmr spectra of charged molecules had been studied before, and a linear correlation was found between the π-electron density on aromatic carbon atoms and the chemical shifts of the aromatic protons attached to these carbons.[6,7] This correlation was extended to ben-

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(1) Correct analyses and spectra were obtained for these compounds.

(2) The chemical shifts were determined relatively to the low-field component of the CH_2 quartet of ether at τ 6.42. The position of this signal was not changed by the presence of butyllithium.

(3) These species are written in the form of anions for convenience sake. The ionic character of allyllithium was proved recently.[4] Only one resonance form is written here.

(4) P. West, J. I. Purmort, and S. V. McKinley, *J. Am. Chem. Soc.*, **90**, 797 (1968).

(5) *cis* and *trans* enynes were obtained. The composition of the products changes with the conditions of the reaction and depends also on what is protonated or silylated: the mono- or the dianion. These results will be reported in a subsequent paper.

Table I.[a] Proton Chemical Shifts (τ)

Compd	A[b]	B	C or R	D	E or R′	Coupling constants, cps
Ia	2.72 (m)	6.35 (d)	4.0	4.9 (m)	8.33 (d)	J_{BC} 4.0
IIa	3.08 (m)	6.16 (d)	4.14 (q)	5.08 (m)	8.42 (d)	J_{CD} 16.5; J_{BC} 10.0; $J_{DR'}$ 7.0
IIIa	3.07 (m)		3.55 (d)	4.81 (m)	*c*	J_{CD} 15.5; $J_{DR'}$ 7.0
Ib	2.63 (m)	6.88 (s)	8.15 (s)	5.11 (d)	4.89 (d)	
IIb	3.12 (m)	6.37 (s)	8.30 (s)	6.26[e]	5.89 (d)	J_{DE} 3
IIIb	3.07–3.18 (m)		7.99 (s)	5.89[e]	5.60[e]	
Ic	2.74 (m)	6.8 (d)	3.88 (se)	3.30 (d)	2.74 (m)	J_{BC} 6; J_{BD} 1.5; J_{CD} 16.0
IIc	3.03–3.10 (m)	6.58 (d)	*d*	4.51 (d)	3.03–3.10 (m)	J_{BC} 9.5; J_{CD} 15.0
IIIc	3.01 (m)		2.22 (d)	3.96 (d)	3.01 (m)	J_{CD} 15.0
Id	2.78 (m)	6.91 (d.d)	3.8–4.5 (m)	4.68 (q)	4.91 (q)	J_{BC} 5; J_{DE} 2; J_{CE} 10; J_{CD} 16.0
IId	3.07 (m)	6.52 (d)	3.76 (se)[f]	5.77 (q)	6.26 (q)	J_{BC} 9.2; J_{DE} 2; J_{CE} 10; J_{CD} 16.2
IIId	3.20 (m)		3.01 (q)	5.36 (d)[e]	5.90 (d)[e]	J_{CE} 9; J_{DC} 16.2
Ie	8.80 (s)	7.19 (s)	8.23 (s)	5.16[e]	5.02[e]	
IIe	*c*	7.08 (s)	8.35 (s)	6.18	6.46 (m)	
IIIe	*c*		8.06 (s)	6.08	6.46 (m)	

[a] s, singlet; d, doublet; q, quartet; m, multiplet; se, sextet. All bands were integrated and corresponded to the number of protons indicated by the formulas. [b] Position of the main peak. [c] Hidden by the signals of the medium. [d] Hidden by the phenyl signals. [e] Broad peak. [f] Degenerate octet.

zyllithium derivatives[8] and their complexes.[9] The charge distribution in the benzylic anions calculated from the proton magnetic shifts showed agreement with SCF but not LCAO calculations.[8] Charge delocalizations, based on the chemical shifts of the protons, were found in allyllithium,[4,10] phenylallyllithium[11] and -potassium,[12] and pentadienyllithium[13] derivatives, but their densities were not quantitatively correlated with the chemical shifts of the protons in view of possible disturbing factors like anisotropy effects. The upfield shift of the two =CH protons at the end of the enyne system on monometalation of I proved that the charge formed on the propargylic carbon is delocalized on the vinyl group. The upfield shift of the terminal vinyl hydrogen in II (0.1–1.1 ppm) relative to I is, however, much less than the corresponding shift in the allylic,[4,10] phenylallylic,[11,12] and pentadienylic[13] anions (1–3 ppm). The change in the chemical shift (0.3–0.5 ppm) of the aromatic protons on monometalation of I is also less than that found in the benzylic[8] and phenylallylic[11,12] anions (1–2 ppm). These results support the view that delocalization of charge is much less in the pentenynylic anion than in the allyl or pentadienyl systems.

The large difference in the downfield shifts of the B protons for the conversion of Ia to IIa and Ib to IIb may be due to conformational differences between Ia and Ib or the two monoanions IIa and IIb. However, this interpretation is doubtful, since proton D suffers a larger upfield shift on the conversion of Ib to IIb or Id to IId than for the transformation of Ia to IIa. The delocalization of the charge is more extensive to the terminal methylene in IIb or IId than to the methyl-substituted C-5 of IIa. This delocalization is expected to be accompanied by a lower charge density, on the carbon holding the B proton, in IIb or IId than in IIa. The large upfield shift of the D proton is not due to conformational effects since a similar shift is observed for the proton E. The coupling constants between the protons B and C in IIa, IIc, and IId seem to indicate a *s-trans* conformation[12,13] around the bond between the carbons to which these protons are attached.

Dimetalation of I produced an even more dramatic effect on the chemical shifts. The terminal vinylic protons, which were expected to move to higher field on introduction of an additional negative charge in a delocalized system, were instead shifted to lower field. This could not be an anisotropy effect alone since the anisotropic groups are removed from these protons and the *cis* and *trans* protons of the terminal vinylic carbon suffer comparable shifts. The downfield shift of the protons D and E observed on transformation of IIe to IIIe is much less than in metalating IIa–d to IIIa–d and suggests the possibility that the conformation of the phenyl ring might be different in these dianions (*e.g.*, perpendicular to the olefin plane) than in the monoanions (IIa–d) (*e.g.*, parallel to the olefin plane). The protons of the phenyl group remain essentially unchanged on transformation of IIa–d to IIIa–d. These effects point to a withdrawal of the charge that was delocalized in the periphery of the monoanion. Both negative charges are apparently localized now on the three-carbon segment formed from the acetylenic and propargylic atoms, apparently because of the special stability of this system of three carbons containing eight electrons and linked to one group at each end. The observed charge localization may also be due in part to ion-pairing changes.[14]

The large downfield shift of proton C and R groups on metalating the mono- into the dianions (0.6–0.75 ppm) is also in line with effects connected with the high electronegativity of the C_3^{2-} group. It is not connected with the anisotropy and changes in the conformation of the phenyl since this effect is also observed in the aliphatic anions IIe and IIIe.

Two structures are conceivable for the dianions III, one (IV) of the allenic type, having two pairs of electrons in two sp^2 orbitals, and a second (V) of the acetylenic type, with three sp-hybridized carbons. It seems to us

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(6) G. Fraenkel, R. E. Carter, A. McLachlan, and J. H. Richards, *J. Am. Chem. Soc.*, **82**, 5846 (1960).

(7) T. Schaefer and W. G. Schneider, *Can. J. Chem.*, 41, 966 (1963).

(8) V. R. Sandel and H. H. Freedman, *J. Am. Chem. Soc.*, **85**, 2328 (1963).

(9) R. Waack and M. A. Doran, *ibid.*, **85**, 4042 (1963).

(10) C. S. Johnson, M. A. Weiner, J. S. Waugh, and D. Seyferth, *ibid.*, **83**, 1306 (1961); D. Seyferth and T. F. Jula, *J. Organometal. Chem.* (Amsterdam), **8**, 913 (1967).

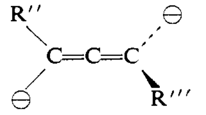
(11) V. R. Sandel, S. V. McKinley, and H. H. Freedman, *J. Am. Chem. Soc.*, **90**, 495 (1968).

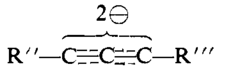
(12) G. J. Heiszwolf and H. Kloosterziel, *Rec. Trav. Chim.*, **86**, 807, 1345 (1967).

(13) R. B. Bates, D. W. Gosselink, and J. A. Kaczynski, *Tetrahedron Letters*, 205 (1967).

(14) R. C. Roberts and M. Szwarc, *J. Am. Chem. Soc.*, **87**, 5542 (1965).

that IV is expected to have a stability not greater or even lower than that of an allyl anion, since there is a poor overlap between the orbitals containing the electron pairs and the π system. We favor structure V, which we submit to call an extended acetylene or a


IV


V

sesquiacetylene.[15] Here we have the unusual occurrence of two identical four-electron three-center systems delocalized symmetrically. The orbitals of each π system, being parallel, have better overlap and permit bond-length adjustment for higher stability.

Extended acetylenes can be metalated further, when a hydrogen is linked to the sp carbons. Thus metalation of propyne[16] yielded C_3Li_4 which would be the parent lithium sesquiacetylide, and prolonged metalation of 1-phenylpropyne[17] gave $C_6H_5C_3Li_3$ or lithium phenylsesquiacetylide. These compounds were identified by the formation of tetrakis(trimethylsilyl) and trideuterio derivatives, respectively.[16,17]

Low delocalization of electrons in the anions II and III is supported by the different chemical shifts of protons C and E in these compounds, revealing a high bond order and slow rotation, on the nmr scale, around the initially double bond. Pentadienylic anions[15] show a faster rotation around this bond at the temperatures of our measurements.

Acknowledgment. We are grateful to Professor J. Musher for an interesting discussion.

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(15) These are extended acetylenes containing a carbon atom in addition to the ordinary two-carbon acetylene group. Other extended acetylenes, containing different atoms, *e.g.*, nitrogen, are conceivable. The dianion formed from a nitrile could be called a sesquiazacetylene

$$-{}^{2-}C-C\equiv N \longleftrightarrow -C\equiv C-N^{2-}$$

(16) R. West, P. A. Carney, and I. C. Mineo, *J. Am. Chem. Soc.*, **87**, 3788 (1965).

(17) J. E. Mulvaney, T. L. Folk, and D. J. Newton, *J. Org. Chem.*, **32**, 1674 (1967).

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Feline Gastrin. An Example of Peptide Sequence Analysis by Mass Spectrometry

Sir:

The potential of mass spectrometry in sequence analysis of peptides has been recognized for some time.[1] However, although considerable success has been achieved with cyclic and antibiotic peptides,[2] there seems to have been no practical use of the technique with more typical peptides derived from the normal protein biosynthetic pathway. This is doubtless because there is a severe limit to the size of peptides amenable to current techniques (of the order of ten residues depending on their composition), and hence mass spectrometry must be applied in conjunction with other methods of peptide analysis. We now report an example of sequence analysis of a mammalian peptide hormone.

The gastrins are heptadecapeptide amides with powerful stimulatory action on the secretion of gastric acid.[3] The structures of gastrins from five mammalian species have been reported,[4] and they display sequence variations corresponding to single-base substitutions in genetic codons. Professor R. A. Gregory recently isolated the gastrin (*ca.* 400 μg) from 200 cat antra and generously gave it to us for structure determination. Amino acid analysis (17 nmol) demonstrated its essential similarity to other members of the gastrin family. The composition $Ala_2Asp_1Gly_2Glu_5Leu_1Met_1Phe_1Pro_1Trp_2Tyr_1$ was compatible with five structures (**1**) corresponding to single base changes in genetic codons, although other possibilities could not be excluded. With the remaining material (180 nmol) it was possible to assign a unique sequence to the hormone by mass spectrometry, without recourse to conventional chemical sequencing methods.

The feline gastrin was treated with 0.1 *N* HCl at 20° for 2 hr to hydrolyze the phenolic sulfate ester[5] and then with chymotrypsin (enzyme:substrate ratio 1:7.5) at pH 8 and 4° for 24 hr. The four chymotryptic peptides were isolated by gel filtration on Sephadex G-25 and then treated as follows. Chym-III was esterified (0.1 *N* methanolic HCl at 20° for 12 hr) and then acetylated (acetic anhydride–acetic acid (1:1) at 20° for 12 hr); direct mass spectrometry sufficed to establish the dipeptide structure (**4**). Chym-IV was treated similarly. Its mass spectrum showed the expected molecular ion, but it was dominated by side-chain fragmentation. The tripeptide structure (**5**) was confirmed by the identity of its spectrum with that of an authentic sample. Chym-I was permethylated[6] to give a product sufficiently volatile for easy obtention of the mass spectrum. Its spectrum contained ions attributable to the tetramethyl derivative **2** together with a pentamethyl derivative in which C-methylation had occurred at the glycine residue.[7] The spectrum

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(1) *E.g.*, K. Biemann, *Chimia*, **14**, 393 (1960); E. Stenhagen, *Z. Anal. Chem.*, **181**, 462 (1961); F. Weygand, A. Prox, H. H. Fessel, and K. K. Sun, *Z. Naturforsch.*, **20b**, 1169 (1965); M. M. Shemyakin, Y. A. Ovchinnikov, A. A. Kiryushkin, E. I. Vinogrodova, A. I. Miroshnikov, Y. B. Alakhov, V. M. Lipkin, Y. B. Shvetsov, N. S. Wulfson, B. V. Rosinov, V. N. Bochkarev, and V. M. Burikov, *Nature*, **211**, 361 (1966).

(2) *E.g.*, M. Barber, P. Jolles, E. Vilkas, and E. Lederer, *Biochem. Biophys. Res. Commun.*, **18**, 469 (1965); A. Prox and F. Weygand, "Peptides," Proceedings of the Eighth European Peptide Symposium, North-Holland Publishing Co., Amsterdam, 1967, p 158; T. Wieland with G. Lüben, H. Ottenheym, J. Faesel, J. X. de Vries, W. Konz, A. Prox, and J. Schmid, *Angew. Chem.*, **80**, 209 (1968).

(3) R. A. Gregory, *Gastroenterology*, **51**, 953 (1966); *Proc. Roy. Soc.* (London), B 170, 81 (1968).

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(5) Both sulfated and unsulfated forms of feline gastrin were isolated from antral mucosa. The former predominated and was used in the present investigation.

(6) *Cf.* B. C. Das, S. D. Géro, and E. Lederer, *Biochem. Biophys. Res. Commun.*, **29**, 211 (1967); D. W. Thomas, B. C. Das, S. D. Géro, and E. Lederer, *ibid.*, **32**, 199 (1968); K. L. Agarwal, R. A. W. Johnstone, G. W. Kenner, D. S. Millington, and R. C. Sheppard, *Nature*, **219**, 498 (1968).

(7) C-Methylation can in general be avoided by reducing the amount of base present in the methylation reaction.