BUO·NHNH₂

↓

BUO·NHN=C(CH₃)₂   IV

| H₂, Pt

↓

BUO·NHNHCH(CH₃)₂   V

Bz
|
H·Tyr·OC₂H₅ ·HBr   II

OBz
[benzene ring]
CH₂

| COCl₂

→ III

OCNCHCO·OC₂H₅ ,

R   Bz
| |
BUO·NHNCO—Tyr·OC₂H₅   1. HCl
VI   2. NaHCO₃ →

R   Bz
| |
H₂NNCO—Tyr·OC₂H₅   CBO·Arg(NO₂)·OH
VIII   DCCI →

NO₂   R   Bz
| | |
CBO·Arg—NHNCO—Tyr·OC₂H₅   NaOH →
IX

NO₂   R
| |
CBO—Arg—NHNCO—Tyr·OH
X

BUO = (CH₃)₃COCO—;

CBO = [benzene ring]—CH₂OCO— ;   Bz = [benzene ring]—CO—;

DCCI = N,N'-dicyclohexylcarbodiimide;   R = $\overset{H_3C}{\underset{H_3C}{\diagdown}}$CH—

methanol–water–chloroform–benzene (3:1:3:1), m.p. 144–146°, $[\alpha]^{24}$D −43°, λ_{max} 271 mμ (ϵ 16,850) (*Anal.* Calcd. for $C_{53}H_{70}N_{14}O_{13}$: C, 57.29; H, 6.32; N, 17.65. Found: C, 57.60; H, 6.50; N, 17.44).

Cleavage of the benzyloxycarbonyl protecting group of XI with hydrobromic acid in acetic acid and subsequent base treatment yielded N-[2-isopropyl-3-(nitro-L-arginyl)-carbazoyl]-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester (XII), m.p. 124–130°, $[\alpha]$D −57° (*Anal.* Calcd. for $C_{55}H_{64}N_{14}O_{11}$: C, 55.31; H, 6.60; N, 20.07. Found: C, 55.32; H, 6.39; N, 19.50), which was condensed with benzyloxycarbonyl L-aspartic acid-β-benzyl ester[13] under the influence of dicyclohexylcarbodiimide to afford N-[2-isopropyl-3-(benzyloxycarbonyl-[[β-benzyl]]-L-aspartyl-nitro-L-arginyl)carbazoyl]-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester (XIII), m.p. 136–142°, $[\alpha]^{25}$D −40° (*Anal.* Calcd. for $C_{64}H_{81}N_{15}O_{16}·H_2O$: C, 57.61; H, 6.27; N, 15.75. Found: C, 57.29; H, 6.35; N, 15.56). Scission of the benzyloxycarbonyl, benzyl ester, and nitro groups of XIII by catalytic hydrogenation and then treatment with concentrated hydrochloric acid at 40° for 1 hr. to remove the methyl ester function[14] provided the free isosteric octapeptide (I). Purification was achieved by countercurrent distribution in the systems n-butyl alcohol–water and sec-butyl alcohol–water to give I as an amorphous solid, m.p. 193–198°, $[\alpha]$D²³ −33° (water). Homogeneity was established by paper electrophoresis[15]

(13) Cyclo Chemical Corp.

(14) R. B. Merrifield and D. W. Woolley, *J. Am. Chem. Soc.*, **78**, 4646 (1956).

(15) A Misco paper electrophoresis apparatus and organic buffers containing 10% urea were used for these experiments as described by L. N. Werum, H. T. Gordon, and W. Thornburg, *J. Chromatog.*, **3**, 125 (1960).

(single spot with K₃Fe(CN)₆–FeCl₃ at pH 4, 7.2, and 8) and paper chromatography[16] (R_f (1) 0.38; R_f (2) 0.30; R_f (3) 0.45; single spot with K₃Fe(CN)₆–FeCl₃ and p-nitrobenzene diazonium fluoroborate and Sakaguchi reagents). Quantitative amino acid determination gave the following molar ratio: Asp, 1.1; Arg, 0.9; Tyr, 0.9; Val, 1.0; His, 1.0; Phe, 1.0; proline was not determined.[12]

Biological activity was evaluated on the isolated rat uterus and through blood pressure measurements in intact, phenobarbital-anesthetized rats. Isostere I has $1/100$th to $1/200$th of the activity of Val⁵-angiotensin II-Asp¹-β-amide (XIV)[17] in these assays and produces a twofold increase in duration of pressor action over XIV in the rat at doses which give an equivalent absolute response. The corresponding isosteric C-terminal hexa- and heptapeptides, synthesized by similar methods, exhibited 0.2% and 50–100%, respectively, of the activity of I.

Structure–activity studies to date have indicated that, in order to be active, analogs of angiotensin II must contain the pentapeptide sequence Tyr-Val (or Ileu)-His-Pro-Phe *plus* at least one additional amino acid attached at the N-terminus. The present results show that peptides in which this amino acid has been replaced with -NHN(R)CO- retain significant biological activity. This suggests that, even in the interior of a peptide chain, the isosteric moiety is able to assume a conformation which resembles that of an amino acid.

The implications of isosteric replacement of amino acids in a peptide chain to such problems as susceptibility to enzymatic degradation will be the subject of a subsequent publication.

Acknowledgment.—We are grateful to Dr. J. W. Constantine of our Pharmacology Department for the biological determinations.

(16) The R_f values (on Whatman paper No. 4) refer to the following paper chromatographic systems: (1) sec-butyl alcohol–formic acid (88%)–water (7:1:2); (2) ethyl acetate–pyridine–water (12:5:4); (3) methyl isobutyl ketone–formic acid (88%)–water (2:1:1).

(17) Hypertensin-Ciba®. This material produced an average increase of 50 mm. in rat blood pressure following intravenous administration of 0.1–0.2 μg./kg. Cf. F. Gross and H. Turrian in "Polypeptides Which Affect Smooth Muscles and Blood Vessels," M. Schachter, Ed., Pergamon Press, New York, N. Y., 1960, p. 137.

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Geminal Proton–Proton Coupling Constants in CH₂=N— Systems[1]

Sir:

It is commonly known[2] that J_{HH}(gem) in the sp²-type CH₂ groups of olefins is usually small in magnitude and can be either positive or negative; there is a fairly good inverse correlation[2h] with the electronegativity (E_X) of the substituent in CH₂=CH—X compounds. These olefinic J_{HH}(gem) values fall out-

(1) Part III of the series "NMR Spectral Studies of sp²-type CH₂ Systems." For Part II, see B. L. Shapiro, R. M. Kopchik, and S. J. Ebersole, *J. Chem. Phys.*, in press.

(2) *E.g.* (a) C. N. Banwell, A. D. Cohen, N. Sheppard, and J. J. Turner *Proc. Chem. Soc.*, 266 (1959); (b) C. N. Banwell and N. Sheppard, *Mol. Phys.*, **3**, 351 (1960); (c) C. N. Banwell, N. Sheppard, and J. J. Turner, *Spectrochim. Acta*, **16**, 794 (1960); (d) E. B. Whipple, J. H. Goldstein, and L. Mandell, *J. Am. Chem. Soc.*, **82**, 3010 (1960); (e) W. Bruegel, Th. Ankel, and F. Krueckeberg, *Z. Elektrochem.*, **64**, 1121 (1960); (f) A. A. Bothner-By and C. Naar-Colin, *J. Am. Chem. Soc.*, **83**, 231 (1961); (g) G. S. Reddy, J. H. Goldstein, and L. Mandell, *ibid.*, **83**, 1300 (1961); (h) T. Schaefer, *Can. J. Chem.*, **40**, 1 (1962); (i) A. A. Bothner-By, C. Naar-Colin, and H. Günther, *J. Am. Chem. Soc.*, **84**, 2748 (1962); (j) G. S. Reddy and J. H. Goldstein, *J. Mol. Spectry.*, **8**, 475 (1962); (k) R. T. Hobgood, Jr., G. S. Reddy, and J. H. Goldstein, *J. Phys. Chem.*, **67**, 110 (1963).

TABLE I

Compound[a]	$J\|_{HH}(gem)^b$ (c.p.s.)	Solvent[c]	$J\|$ "average"[d] (c.p.s.)
CH₂=N—OH (or D)	7.63 to 9.95[e]	[e]	} 8.5
CH₂=N—OCH₃	6.96 to 9.22[e]	[e]	
CH₂=N—N(CH₃)₂	10.3 ± 0.2	50% in D₂O	
	11.7 to 12.0 ± 0.2	C₆H₆[f]	
CH₂=N—N—C₆H₄—NO₂(4)	11.6 ± 0.2	Me₂CO, Me₂SO	
|			
H			
CH₂=N—N—C₆H₃—(NO₂)₂(2,4) (or D)	11.0 to 11.4[g]	[g]	} 11
|			
H			
(or D)			
CH₂=N—N—C₆H₄—NO₂(4)	12. ± 0.2	Me₂CO	
|			
CH₃			
CH₂=N—C(CH₃)₃	16.11	MeCN	
	16.52	(neat)	
CH₂=N—C(CH₃)₂CH₂C(CH₃)₃[h]	16.08	Me₂SO	} 16.5
	16.20	MeCN	
	16.34	ClCH₂CH₂Cl	
	16.86	CCl₄	
	16.97	(neat)	

[a] All compounds were prepared in standard fashion by the reaction of formaldehyde with the appropriate H₂N–R compound. [b] All J values were measured on carefully calibrated Varian Associates Model A-60 spectrometers, radiofrequency 60 Mc./sec., sample temp. 36 ± 2°. Unless otherwise specified, the P.E. of the values given is 0.05 c.p.s. or less. [c] Unless otherwise noted, dilute (~5% or less) solutions were used. [d] A rough "average" value, convenient for indicating the general sizes of the couplings. [e] Strongly solvent dependent. For a more detailed account, see B. L. Shapiro, S. J. Ebersole, and R. M. Kopchik, J. Mol. Spectry., 11, 326 (1963). [f] Small concentration dependence. [g] Small solvent dependence. See G. J. Karabatsos, B. L. Shapiro, F. M. Vane, J. S. Fleming, and J. S. Ratka, J. Am. Chem. Soc, 85, 2784 (1963). [h] We thank Dr. P. L. de Benneville of the Rohm and Haas Co., Bristol, Pa., for generous samples of this compound and for helpful discussions.

side the range 0 ± 3.5 c.p.s. only for the cases of substitution by elements of very low electronegativity, such as Al, Li, and Mg,[3] where values in the range (+) 6 to 8 c.p.s. are observed. In Table I, we report our observations of $|J_{HH}|(gem)$ in a number of CH₂=N— compounds, in which very different values are obtained.

Among other interesting features[4] of the spectra, the magnitudes and (in some cases) solvent dependences[4] of the J values are noteworthy. In these CH₂=N—Y compounds, $|J|$ increases markedly as the electronegativity of Y decreases. The trend observed here, taken together with that reported for the olefins,[2h] suggests that the sign of these CH₂=N—Y J values is negative, and various sign determination experiments are in progress.

It may be noted at this stage, however, that the range of magnitudes of these sp²-type J(gem) values overlaps extensively with the range of values hitherto associated with sp³-type J(gem) values.[5] Thus, regardless of the signs of the J values reported here, a theoretical picture which gives major importance to the H–C–H bond angles[6] will clearly be inapplicable, since it is evident that in our sp²-type cases, substituent effects (to put the matter in the most general terms) are dominant, as has already been pointed out for sp³-type CH₂ systems and suggested strongly for vinylic sp² cases as well.[7]

Regardless of the detailed nature of the dominant factors controlling the spin–spin coupling constants, the observed monotonic trend in $|J|(gem)$ with β-

(3) (a) D. W. Moore and J. A. Happe, J. Phys. Chem., 65, 224 (1961); (b) C. S. Johnson, Jr., M. A. Weiner, J. S. Waugh, and D. Seyferth, J. Am. Chem. Soc., 83, 1306 (1961); (c) R. T. Hobgood, Jr., and J. H. Goldstein, Spectrochim. Acta, 18, 1280 (1962); (d) G. Fraenkel, D. G. Adams, and J. Williams, Tetrahedron Letters, No. 12, 767 (1963).

(4) To be discussed at length elsewhere.

(5) See for example, M. Barfield and D. M. Grant, J. Am. Chem. Soc., 86, 1899 (1963), and references cited therein.

(6) Cf. M. Karplus and D. H. Anderson, J. Chem. Phys., 30, 6 (1959); H. S. Gutowsky, M. Karplus, and D. M. Grant, ibid., 31, 1278 (1959); H. S. Gutowsky, V. D. Mochel, and B. G. Somers, ibid., 36, 1153 (1962); M. Karplus, J. Am. Chem. Soc., 84, 2458 (1962).

(7) H. J. Bernstein and N. Sheppard, J. Chem. Phys., 37, 3012 (1962).

atom electronegativity (cf. vinyl-X[2h]) suggests that resonance form B, which is certainly unimportant in the azomethines, is also probably not very significant in the oxime and hydrazone derivatives. Resonance contributors of type A, however, could well be involved to some significant extent in all three types of compound. One possible and plausible implication of

$$\overset{+}{CH_2}{-}\overset{-}{N}{-}Y \qquad \overset{-}{CH_2}{-}\overset{+}{N}{=}Y$$
$$\quad A \qquad\qquad\qquad B$$

this is that the more "positive" the carbon atom of the CH₂, the larger the $|J|$. However, other factors (such as the involvement of the π-system, and especially the availability of electron pairs on X of the CH₂=X system) may well be important. These and other matters are currently being investigated.

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Complexes of Organolithium Compounds with Vacant Orbital Acceptors. II. Determination of Electron-Density Changes by Proton Magnetic Resonance

Sir:

We wish to report that changes in electron density of an aromatic organolithium compound, brought about by dative-bond formation with a Lewis acid, can be determined from changes occurring in its proton magnetic resonance (p.m.r.) spectra on complexing. Organolithium compounds form reversible dative complexes