The attachment of a chromophore to the deoxyribose could contribute an additional rotation to the levorotatory sugar. Thus the positive Cotton effect of C seems to be strong enough to cause a net dextrorotation in the visible region. This is not the case for T, the rotation of which should be very small above 350 mμ. On the other hand, A and G are expected to be even more levorotatory than deoxyribose in the visible region, a conclusion in accord with the recent data of Ts'o, *et al.*[11]

Since the helical structure of DNA consists of A–T and G–C base pairs, we have included in the figures the calculated rotations of such combinations. Note that the G–C curve dominates the ORD throughout the Cotton effect region, as compared with the extremely small net rotation of the A–T curve over the same region. Thus, the 290-mμ peak in DNA[6] depends at least partially on the cytosine content of the species, although the rotations of the mononucleotides could vary when incorporated into a polynucleotide chain. To what extent the sequence and stacking interactions of the base pairs in the helical structure of nucleic acids will affect the Cotton effects is a subject for future investigations. Indeed, ORD promises a quantitative approach to the study of the structures of synthetic polynucleotides and natural nucleic acids.

Acknowledgment.—We thank Professor I. Tinoco, Jr., for his valuable discussions.

(11) P. O. P. Ts'o, G. K. Helmkamp, and C. Sander, *Biochim. Biophys. Acta*, **55**, 584 (1962).

(12) Helen Hay Whitney Foundation Fellow.

CARDIOVASCULAR RESEARCH INSTITUTE
UNIVERSITY OF CALIFORNIA
SAN FRANCISCO 22, CALIFORNIA

JEN TSI YANG
TATSUYA SAMEJIMA[12]

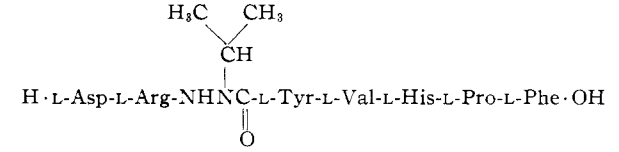
RECEIVED AUGUST 31, 1963

N-[2-Isopropyl-3-(L-aspartyl-L-arginyl)-carbazoyl]-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalanine,[1] an Isostere of Bovine Angiotensin II

Sir:

A great many structural modifications have been explored in a number of biologically-active polypeptides such as the hypothalamo-neurohypophysial hormones, oxytocin and vasopressin,[2] the peptides derived from blood plasma, angiotensin,[3] and bradykinin,[4] and more recently the endecapeptide, eledoisin.[5] While these studies have been concerned largely with the substitution of one amino acid for another, isosteric[6] alteration of amide linkages *within* a polypeptide and the resultant effects on activity and biological stability have not been explored.

We wish to report the first example of a polypeptide with biological activity which has an isosteric unit in an internal position of the polyamide chain. This substance, an analog of bovine angiotensin II[7] containing a carbazoyl unit, is represented by formula I and has been synthesized as follows. Reaction of O-benzoyl-



L-tyrosine ethyl ester hydrobromide (II), m.p. 223–225°, $[\alpha]^{24}D$ +11° (*Anal.* Calcd. for $C_{18}H_{20}BrNO_4$: C, 54.83; H, 5.11; Br, 20.27; N, 3.55. Found: C, 54.89; H, 5.20; Br, 20.08; N, 3.44),[8] with phosgene in toluene at 150°[9] resulted in the formation of N-carbonyl-O-benzoyl-L-tyrosine ethyl ester (III), which was treated with *t*-butyl-3-isopropylcarbazate (V), m.p. 47–51° (*Anal.* Calcd. for $C_8H_{18}N_2O_2$: C, 55.14; H, 10.41; N, 16.08. Found: C, 55.01; H, 10.25; N, 16.06), to afford N-[2-isopropyl-3-(*t*-butyloxycarbonyl)carbazoyl]-O-benzoyl-L-tyrosine ethyl ester (VI), m.p. 126–127°, $[\alpha]^{24}D$ +6° (*Anal.* Calcd. for $C_{27}H_{35}O_7N_3$: C, 63.14; H, 6.87; N, 8.18. Found: C, 63.40; H, 7.00; N, 8.33). The *t*-butyl 3-isopropylcarbazate (V) needed for this synthesis was obtained by catalytic reduction of *t*-butyl isopropylidenecarbazate (IV), m.p. 104–105° (*Anal.* Calcd. for $C_8H_{16}N_2O_2$: C, 55.79; H, 9.36; N, 16.27. Found: C, 55.94; H, 9.12; N, 16.48), which, in turn, was prepared from *t*-butyl carbazate[10] and acetone. Removal of the *t*-butyloxycarbonyl protecting group of VI with hydrochloric acid gave the crystalline hydrochloride VII, m.p. 137–138°, $[\alpha]^{24}D$ −7° (*Anal.* Calcd. for $C_{22}H_{28}ClN_3O_5$: C, 58.73; H, 6.27; Cl, 7.88; N, 9.34. Found: C, 59.00; H, 6.35; Cl, 7.81; N, 9.57), which was converted with alkali to N-[2-isopropylcarbazoyl]-O-benzoyl-L-tyrosine ethyl ester (VIII), m.p. 147–148°, $[\alpha]^{22}D$ +20° (*Anal.* Calcd. for $C_{22}H_{27}O_5N_3$: C, 63.90; H, 6.58; N, 10.16. Found: C, 63.73; H, 6.59; N, 9.97). Condensation of VIII with benzyloxycarbonylnitro-L-arginine[11] by the dicyclohexylcarbodiimide method furnished crystalline N-[2-isopropyl-3-(benzyloxycarbonylnitro-L-arginyl)carbazoyl]-O-benzoyl-L-tyrosine ethyl ester (IX), m.p. 157–159°, $[\alpha]^{22}D$ −9°, $\lambda\lambda_{max}$ 231, 268 mμ (ε 22,050, 17,800) (*Anal.* Calcd. for $C_{36}H_{45}N_8O_{10}$: C, 57.66; H, 6.04; N, 14.97. Found: C, 57.46; H, 5.99; N, 14.66). Alkaline hydrolysis of the latter gave crystalline N-[2-isopropyl-3-(benzyloxycarbonylnitro-L-arginyl)-carbazoyl]-L-tyrosine (X), m.p. 116–126°, $[\alpha]^{24}D$ −16°, $\lambda\lambda_{max}$ 225, 271 mμ (ε 13,000, 16,900) (*Anal.* Calcd. for $C_{27}H_{36}O_9N_8$: C, 52.59; H, 5.89; N, 18.17. Found: C, 52.53; H, 5.81; N, 18.10). Coupling of X with L-valyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester[12] by the carbodiimide procedure produced N-[2-isopropyl-3-(benzyloxycarbonylnitro-L-arginyl)carbazoyl]-L-tyrosyl-L-valyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester (XI). This material was purified by countercurrent distribution in the system

(1) The name carbazoyl has been adopted for $H_2NNH \cdot CO-$ in analogy to carbamoyl for $H_2N \cdot CO-$.

(2) See, for example: (a) R. A. Boissonnas, S. Guttmann, B. Berde, and H. Konzett, *Experientia*, **17**, 377 (1961); (b) W. D. Cash, L. M. Mahaffey, A. S. Buck, D. E. Nettleton, Jr., C. Romas, and V. du Vigneaud, *J. Med. Pharm. Chem.*, **5**, 413 (1962); (c) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Biol. Chem.*, **237**, 1563 (1962); (d) C. H. Schneider and V. du Vigneaud, *J. Am. Chem. Soc.*, **84**, 3005 (1962); (e) D. B. Hope and V. du Vigneaud, *J. Biol. Chem.*, **237**, 3146 (1962).

(3) (a) R. Schwyzer, *Helv. Chim. Acta*, **44**, 667 (1961); (b) F. M. Bumpus, P. A. Khairallah, K. Arakawa, I. H. Page, and R. R. Smeby, *Biochim. Biophys. Acta*, **46**, 38 (1961); (c) I. H. Page and F. M. Bumpus, *Physiol. Rev.*, **41**, 331 (1961); (d) K. Arakawa, R. R. Smeby, and F. M. Bumpus, *J. Am. Chem. Soc.*, **84**, 1424 (1962); (e) J. H. Seu, R. R. Smeby, and F. M. Bumpus, *ibid.*, **84**, 3883, 4948 (1962).

(4) (a) R. A. Boissonnas, S. Guttmann, and P. A. Jaquenoud, *Helv. Chim. Acta*, **43**, 1349 (1960); (b) J. Pless, E. Stürmer, S. Guttmann, and R. A. Boissonnas, *Helv. Chim. Acta*, **45**, 394 (1962); (c) E. D. Nicolaides, H. A. De Wald, and M. K. Craft, *Ann. N. Y. Acad. Sci.*, **104**, 15 (1963); (d) M. Bodanszky, M. A. Ondetti, J. T. Sheehan, and S. Lande, *ibid.*, **104**, 24 (1963).

(5) B. Camerino, G. De Caro, R. A. Boissonnas, E. Sandrin, and E. Stürmer, *Experientia*, **19**, 339 (1963).

(6) See V. B. Schatz, in "Medicinal Chemistry," A. Burger, Ed., Interscience Publishers, Inc., New York, N. Y., 1960, p. 72.

(7) The amino acid sequence of bovine angiotensin II is H·L-Asp-L-Arg-L-Val-L-Tyr-L-Val-L-His-L-Pro-L-Phe·OH.

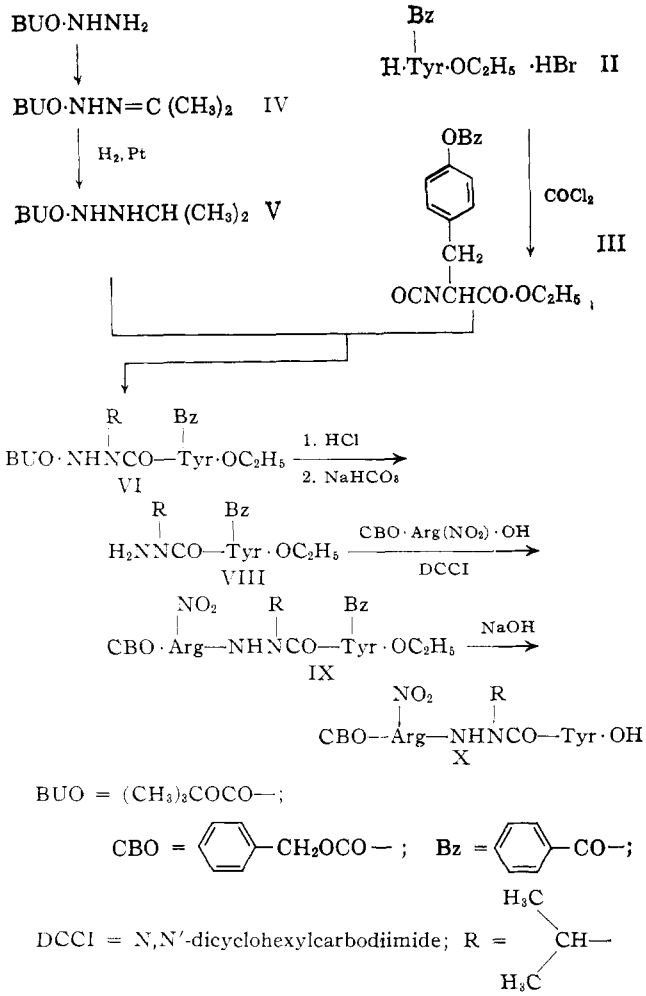
(8) Melting points were taken using a Thomas–Hoover capillary melting point apparatus and are corrected, rotations are in 1% ethanolic solution unless stated otherwise, ultraviolet spectra were measured in methanolic solution.

(9) S. Goldschmidt and M. Wick, *Ann.*, **575**, 217 (1952).

(10) L. A. Carpino, *J. Am. Chem. Soc.*, **79**, 98 (1957).

(11) K. Hofmann, W. D. Peckham, and A. Rheiner, *ibid.*, **78**, 238 (1956).

(12) R. Schwyzer, B. Iselin, H. Kappler, B. Riniker, W. Rittel, and H. Zuber, *Helv. Chim. Acta*, **41**, 1287 (1958).



BUO = $(CH_3)_3COCO-$;

CBO = ; Bz =

DCCI = N,N′-dicyclohexylcarbodiimide; R =

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 $C_{64}H_{81}N_{15}O_{16}\cdot H_2O$: 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°, $[\alpha]D^{23}$ −33° (water). Homogeneity was established by paper electrophoresis[15] (single spot with $K_3Fe(CN)_6$–$FeCl_3$ 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 $K_3Fe(CN)_6$–$FeCl_3$ 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 $^1/_{100th}$ to $^1/_{200th}$ of the activity of Val[5]-angiotensin II-Asp[1]-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.

(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).

(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.

MEDICAL RESEARCH LABORATORIES
CHAS. PFIZER AND CO., INC.
GROTON, CONNECTICUT
HANS-JÜRGEN HESS
WALTER T. MORELAND
GERALD D. LAUBACH

RECEIVED OCTOBER 2, 1963

Geminal Proton–Proton Coupling Constants in $CH_2{=}N-$ Systems[1]

Sir:

It is commonly known[2] that J_{HH}(gem) in the sp^2-type CH_2 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 $CH_2{=}CH{-}X$ compounds. These olefinic J_{HH}(gem) values fall out-

(1) Part III of the series "NMR Spectral Studies of sp^2-type CH_2 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).