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## Optical Rotatory Dispersion of Deoxyribonucleotides[1]

*Sir:*

The optical rotatory dispersion (ORD) of mononucleotides reveals considerable detail not evident in the corresponding absorption spectra. The magnitude of the peak and trough and the sign of the Cotton effect in the ultraviolet region vary significantly among the four deoxyribonucleotides (5′) we studied.

*d*AMP (A), *d*CMP (C), *d*GMP (G), and *d*TMP (T) exhibit only a *single* Cotton effect (between 220 and 380 mμ) with cross-overs (zero rotation) corresponding to their absorption maxima (Fig. 1 and 2),[2-5] as contrasted with the *multiple* Cotton effects of DNA.[6] (Our ORD results of DNA (above 190 mμ) show two peaks at 290 and 223 mμ, two troughs at 255 and 192 mμ, and cross-overs at about 273, 243, and 201 mμ, the magnitude of the second peak and trough being at least twice that of the first one. Although our findings differ in certain aspects from those of Fresco, *et al.*,[6] we agree with them that the multiple Cotton effects, especially the 201-mμ effect in our case, are highly sensitive to the secondary structure of DNA.)

The pyrimidine nucleotides (C and T) have a *positive* Cotton effect as does DNA near 273 mμ, but the purine ones (A and G) actually show a *negative* Cotton effect. Since the bases attached to the deoxyribose have little freedom of rotation and presumably have the same orientation relative to the sugar, this

(1) This work was aided by grants from the U. S. Public Health Service (GM-K3-3441, GM-10880, and HE-06285).

(2) *d*CMP, *d*GMP, and *d*TMP were purchased from the California Corp. for Biochemical Research, and *d*AMP was from the Pabst Laboratories. All ORD measurements were made with a Rudolph manual spectropolarimeter (Model MSP-4); detail of the instrument calibration has been published elsewhere.[3] The concentrations of the solutions were so adjusted that the maximum absorbance was always less than 2. No concentration dependence of the rotations could be detected within normal experimental errors.

(3) J. T. Yang and T. Samejima, *J. Biol. Chem.*, **238**, 3262 (1963).

(4) By assuming a gaussian curve for the circular dichroism and using the Kronig-Kramers transform,[5] the rotational strengths of *d*AMP, *d*CMP, *d*GMP, and *d*TMP were estimated to be approximately −3, +10, −4, and +2 × 10⁻⁴⁰, respectively. These estimates were of course not unique in the absence of circular dichroism measurements and therefore should be viewed with reservation.

(5) A. Mascowitz in "Optical Rotatory Dispersion," C. Djerassi, Ed., McGraw-Hill Book Co., New York, N. Y., 1960, Chapter 12.

(6) J. R. Fresco, A. M. Lesk, R. Gorn, and P. Doty, *J. Am. Chem. Soc.*, **83**, 3155 (1961).
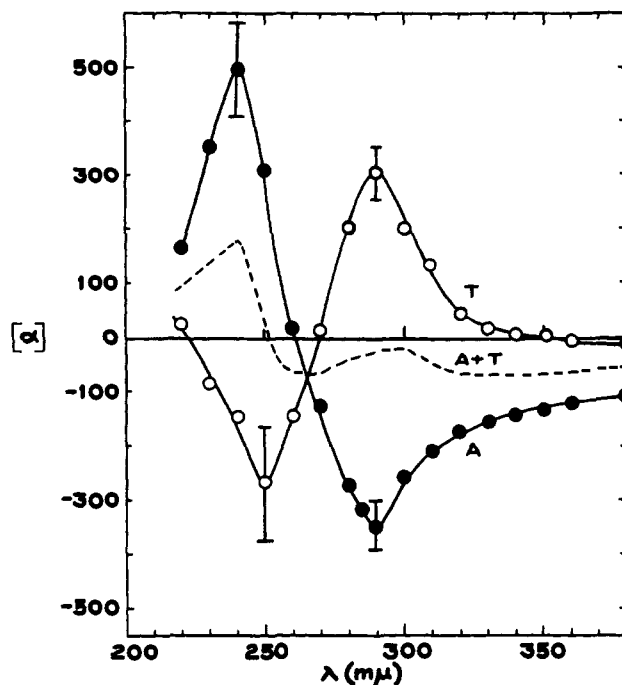


Fig. 1.—Ultraviolet rotatory dispersion of *d*AMP (A) and *d*TMP (T) in 0.15 *M* KF (pH 7.7–7.8). Broken line, $f_A[\alpha]_A + f_T[\alpha]_T$ (*f*'s are the mole fractions).
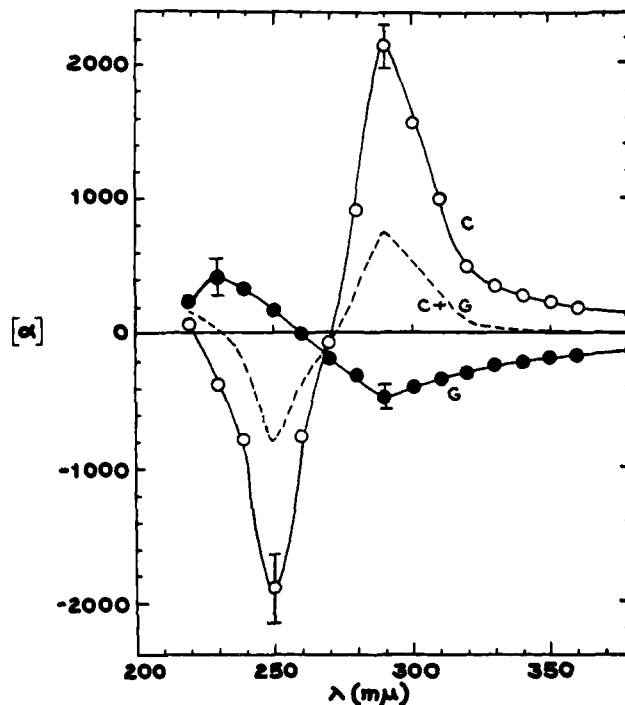


Fig. 2.—Ultraviolet rotatory dispersion of *d*CMP (C) and *d*GMP (G) in 0.15 *M* KF (pH 7.7–7.8). Broken line, $f_C[\alpha]_C + f_G[\alpha]_G$ (*f*'s are the mole fractions). Note that the ordinate scale is different from Fig. 1.

strikingly different rotatory behavior is perhaps related to the directions of the transition moments in the bases.[7,8] To what extent the π–π* transitions[7] and n–π* transitions[9] contribute to the Cotton effect still cannot be answered; indeed, a satisfactory explanation of these opposite Cotton effects is still lacking at present.[10]

(7) H. DeVoe and I. Tonoco, Jr., *J. Mol. Biol.*, **4**, 518 (1962).

(8) R. F. Stewart and N. Davison, *J. Chem. Phys.*, **39**, 255 (1963).

(9) A. Rich and M. Kasha, *J. Am. Chem. Soc.*, **82**, 6197 (1960).

(10) I. Tinoco, Jr., private communication.

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 m$\mu$. 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-m$\mu$ 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.

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# 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

(1) The name carbazoyl has been adopted for H$_2$NNH·CO– in analogy to carbamoyl for H$_2$N·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.

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-

$$\begin{array}{c} \text{H}_3\text{C} \quad \text{CH}_3 \\ \diagdown \quad \diagup \\ \text{CH} \\ | \\ \text{H·L-Asp-L-Arg-NHNC-L-Tyr-L-Val-L-His-L-Pro-L-Phe·OH} \\ \| \\ \text{O} \end{array}$$

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$_8$H$_{18}$N$_2$O$_2$: 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$_7$N$_3$: 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$_8$H$_{16}$N$_2$O$_2$: 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$_3$O$_5$: 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$_5$N$_3$: 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 m$\mu$ ($\epsilon$ 22,050, 17,800) (Anal. Calcd. for C$_{36}$H$_{45}$N$_8$O$_{10}$: 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 m$\mu$ ($\epsilon$ 13,000, 16,900) (Anal. Calcd. for C$_{27}$H$_{36}$O$_9$N$_8$: 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

(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).