TABLE I

ELUTION OF PROTEINS BY CARBON DIOXIDE

100 mg. of exchanger, 10 mg. of protein in 10 ml. of H_2O; washed with 10 ml. of H_2O; and eluted with 10 ml. of H_2O in equilibrium with 1 atm. of CO_2.

	% Sorbed by exchanger	% Desorbed by CO_2[a]
Hemoglobin[b]	60	35
Serum Albumin[c]	25	25
Egg Albumin[d]	60	10
Catalase[e]	30	45
Cathepsin[f]	25	40
Pepsin[g]	30	<5
Casein	75	10
Lysozyme[h]	0	...
Gamma Globulin[i]	60	85
Nucleic Acids[j]	40	0

[a] Calculated from the amount sorbed on the exchanger. [b] Bovine, Armour. [c] Bovine plasma Fraction V, Armour. [d] Crystallized, Armour. [e] Bovine liver, Armour. [f] Bovine kidney, J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **130**, 19 (1939). [g] Porcine crystallized, Armour. [h] Crystallized, Armour. [i] Porcine plasma Fraction II, Armour, [j] Yeast, Schwarz.

Fractionation of kidney cathepsin (Fig. 1) and liver catalase on the exchanger in a column resulted in a two- to ten-fold purification on distilled water–carbon dioxide elution, with a recovery of 90–100% of the total enzymatic activity in each case. The extent of purification of these proteins depended on the purity of the starting material. Porcine plasma Fraction II was also purified by this method.

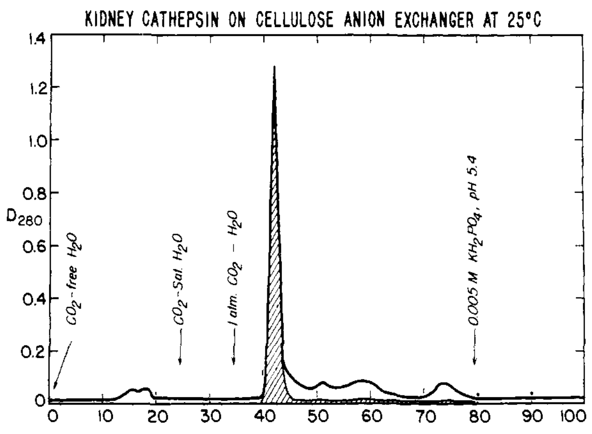

Fig. 1.—Eight grams exchanger (free-base form) in 50 × 1.1 cm. column; load in 5.5 ml.: 250 mg. of $(NH_4)_2SO_4$-fractionated cathepsin dialyzed against CO_2-free water. Fraction volume, 4 ml.; flow rate, 0.5 ml./min. Shaded area represents proportion of catheptic activity in protein fractions. Specific activity of the preparation by Anson's hemoglobin assay, 0.08 ΔD_{280}/mg. protein/10 min. Maximum specific activity of 0.5 was in fraction 42. More than half of the protein remained on the column after all the activity had been eluted.

After carbon dioxide elution, proteins which remain on the column may be subjected to further fractionation by other methods.[2]

RESEARCH DIVISION
ARMOUR AND COMPANY
CHICAGO 9, ILL.
MILTON A. MITZ
SAM S. YANARI

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THE OPTICAL ROTATORY POWER OF POLYAMINO ACIDS AND PROTEINS

Sir:

In a recent article,[1] we derived an expression for the optical rotatory power of an infinitely long helical molecule. Our result for polyglycine in the conformation of a right-handed alpha helix may be generalized for an arbitrary polyamino acid $(-NH-CRH-CO)_n$ in the form

$$[M]_D = [M_0]_D + 49.4(n^2 + 2)/3$$
$$[\alpha]_D = [\alpha_0]_D + 4940(n^2 + 2)/3M \qquad (1)$$

where $[M_0]_D$ and $[\alpha_0]_D$ are the intrinsic residue and the specific rotations of the monomer for the sodium D line, M the residue molecular weight, and n the refractive index of the solvent. We have neglected the effect of vicinal interactions of the residue side chains on the rotation. Under this approximation, the destruction of the right-handed helical conformation should result in a decrease in specific rotation given by

$$-\Delta[\alpha]_D = +4940(n^2 + 2)/3M \qquad (2)$$

If we assume that the L-amino acid residues of a representative natural protein of average residue molecular weight 120 form a right-handed alpha helix, reversible or irreversible denaturation in water, involving destruction of the helical conformation, should lead to a decrease in specific rotation of 52°. The specific rotations of many proteins decrease by approximately this amount upon denaturation.[2]

From equation (2), we predict that the destruction of a right-handed helical structure in poly-γ-benzyl-L-glutamate (PBG) in 20:80 ethylene dichloride–dichloroacetic acid ($n \sim 1.45$) would result in a decrease in specific rotation of 31°. This value agrees well with the experimental decrease of 28° observed by Doty and Yang.[3] Equation (2) predicts a decrease of 49° for the destruction of the helical configuration of poly-L-glutamic acid (PGA) in water. This change would be expected in passing from solutions of low *p*H to those of high *p*H, in which the negative charges of the carboxyl groups would extend the chain. This value is in semi-quantitative agreement with the change of 75° observed by Blout and Idelson.[4] A part of this change is unquestionably due to the direct effect of *p*H on the intrinsic residue rotations.

Since the observed contributions of the helical conformation to the specific rotations of both PBG and PGA are positive, we conclude that both polypeptides are right-handed helices. Our conclusion is in agreement with Huggins'[5] calculations, which indicate that the right-handed helix is the more stable conformation for polymers of L-amino acids.

On the basis of his equivalent theory of optical rotation, Moffitt[6] has predicted for helical molecules a special type of anomalous rotatory dispersion of the form

$$\Delta[\alpha] = -B\nu^2/(\nu_0^2 - \nu^2)^2 \qquad (3)$$

(1) D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* (*Wash.*), **42**, 33 (1956).

(2) C. Cohen, *Nature*, **175**, 129 (1955).

(3) P. Doty and J. T. Yang, THIS JOURNAL, **78**, 497 (1956).

(4) E. R. Blout and M. Idelson, *ibid.*, **78**, 498 (1956).

(5) M. L. Huggins, *ibid.*, **74**, 3963 (1952).

(6) W. Moffitt, private communication. The theory is contained in a forthcoming article in the *Journal of Chemical Physics*.

where B is a constant, ν is the frequency of the incident plane polarized light, and ν_0 is the frequency of the electronic transition making the dominant contribution. At Moffitt's suggestion, Doty and Yang[3] have measured the rotatory dispersion of PBG, confirming Equation (3). We wish to remark that our theory also predicts the same type of anomalous rotatory dispersion. Thus, the rotatory power is proportional to $\alpha_1^2\beta^2$ or $(\alpha_{\|} - \alpha_{\perp})^2$ where $\alpha_{\|}$ and $\alpha_{\perp}$ are the residue polarizabilities parallel and perpendicular to the direction of the helix.[7] If the quantity $\alpha_1\beta$ can be represented by a single Drude dispersion term, $f_0/(\nu_0^2 - \nu^2)$, Equation (3) is at once obtained.

(7) J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479 (1937).

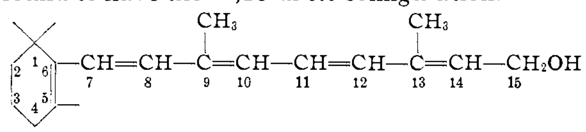
STERLING CHEMISTRY LABORATORY
YALE UNIVERSITY
NEW HAVEN, CONNECTICUT
DONALD D. FITTS
JOHN G. KIRKWOOD

RECEIVED MAY 2, 1956

THE SYNTHESIS AND CONFIGURATION OF NEO-b VITAMIN A AND NEORETINENE b

Sir:

We wish to report that neo b vitamin A and neoretinene b have now been obtained by a synthetic route which establishes their configuration as 11-mono-*cis*.[1] A previously reported 11-*cis* vitamin A,[2] synthesized by the same route, has now been found to have the 11,13-di-*cis* configuration.



Allylic rearrangement of methylvinylethynylcarbinol with moderately strong acid at 80° yields a mixture of the two isomeric 3-methylpent-3-en-1-yn-5-ols.[5] The predominant isomer (**a**),[4] hitherto presumed *trans*,[3,5] showed after distillation through a 100-plate column: b.p. 65° (9.4 mm.), n^{20}D 1.4820, λ_{max} 223 mμ (ϵ 11,000); C–O stretching band 9.92 μ (ϵ 139)[6] (*Anal.* Calcd. for C_6H_8O: C, 74.97; H, 8.39. Found: C, 75.13; H, 8.50); *p*-nitrobenzoate, m.p. 61–62°.

The other isomer (**b**),[4] purified through its *p*-nitrobenzoate, showed: b.p. 73° (9.4 mm.), n^{20}D 1.4934, λ_{max} 224 mμ (ϵ 13,100); C–O stretching band 9.94 μ (ϵ 81) (*Anal.* Found: C, 75.11; H, 8.46). *p*-Nitrobenzoate, m.p. 63–64°; the mixed *p*-nitrobenzoates melted at 50–55°.

Catalytic semihydrogenation yielded the corresponding 3-methylpentadienols, which were acetylated at 25° with acetic anhydride in pyridine. The acetate from **a** *did not add* maleic anhydride at room temperature, while that from **b** gave a 60–70% yield of adduct under the same conditions (*Anal.* Calcd. for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92. Found: C, 60.35; H, 6.01). *This establishes* **a** *as the cis isomer and* **b** *as the trans isomer, contrary to previous assumptions.*

The Grignard reagent from **a** condensed with the "C_{14} aldehyde" to yield the known corresponding glycol[7]; white crystals, m.p. 58°, λ_{max} 229 mμ (ϵ 14,800); C–O stretching band 10.00 μ (ϵ 242). In view of the configuration of **a**, this known glycol must have a 13-*cis* bond. Similarly, **b** was converted to the 13-*trans* isomer, an oil, purified and demonstrated as homogeneous by alumina chromatography: λ_{max} 230 mμ (ϵ 16,700), C–O stretching band at 9.93 μ (ϵ 196) (*Anal.* Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.42; H, 9.96).

Monoacetylation of each glycol, followed by dehydration (tosic acid in benzene), mild hydrolysis, and alumina chromatography, gave in 40–50% over-all yield the corresponding isomer of 11-dehydrovitamin A, a deep yellow oil. In each case only one stereoisomer was produced: 13-*cis:* λ_{max} 317 mμ (ϵ 32,000), C–O stretching band 10.04 μ (ϵ 183), *trans* —CH=CH— band 10.35 μ (ϵ 176) (*Anal.* Calcd. for $C_{20}H_{28}O$: C, 84.45; H, 9.92. Found: C, 84.47; H, 10.06). β-Anthraquinonecarboxylate (cream-white) m.p. 111–112°, $\lambda\lambda_{max}^{cyclohex}$ 256 mμ (ϵ 62,700), 321 mμ (ϵ 38,100). 13-*trans:* λ_{max} 317 mμ (ϵ 34,500), C–O stretching band 9.95 μ (ϵ 112), *trans* —CH=CH— band 10.36 μ (ϵ 182) (*Anal.* Found: C, 84.33; H, 10.21). β-Anthraquinonecarboxylate (deep golden-yellow), m.p. 113.5–115°, $\lambda\lambda_{max}^{cyclohex}$ 256 mμ (ϵ 63,000), 321.5 mμ (ϵ 40,500). A mixture of the two anthraquinonecarboxylates melted at 90–95°.

Catalytic semihydrogenation of 11-dehydro-13-*cis*-vitamin A gave 11,13-di-*cis* vitamin A, the 311-mμ isomer reported previously.[2] The yield of chromatographically purified product, a viscous golden-yellow oil, was 50%; λ_{max} 311 mμ (ϵ 26,000); C–O stretching band 10.03 μ (ϵ 158); *trans* —CH=CH— bond 10.34 μ (ϵ 180) (*Anal.* Calcd. for $C_{20}H_{30}O$: C, 83.86; H, 10.56. Found: C, 83.72; H, 10.62.) First-order rate constant with excess maleic anhydride in ether at 25°: 0.0054/hr. *p*-Phenylazobenzoate, m.p. 99°. Iodine isomerization in the dark produced all-*trans* vitamin A, λ_{max} 325 mμ.[8] Oxidation with manganese dioxide gave the aldehyde, which showed no capacity to produce rhodopsin when treated in the dark with opsin.

Catalytic semihydrogenation of 11-dehydro-13-*trans*-vitamin A gave 11-mono-*cis* vitamin A. The yield of chromatographically purified product, a viscous yellow oil, was 40%; λ_{max} 321 mμ (ϵ 32,500); C–O stretching band 10.08 μ (ϵ 110); *trans* —CH=CH— band 10.35 μ (ϵ 191) (*Anal.* Found: C, 83.22; H, 10.67). First-order rate constant with excess maleic anhydride, same conditions as above: 0.03/hr. *p*-Phenylazobenzoate, m.p. 67°. Iodine isomerization in the dark produced all-*trans* vitamin A, λ_{max} 325 mμ.[8] The ultraviolet and infrared absorption curves of 11-mono-*cis* vitamin A were identical with those of an authentic specimen of neo b vitamin A.[9]

(1) The numbering system used here corresponds to that officially adopted for carotenoids: *Chem. and Eng. News*, **24**, 1235 (1946).

(2) G. Wald, P. K. Brown, R. Hubbard and W. Oroshnik, *Proc. Nat. Acad. Sci.*, **41** (7), 438 (1955).

(3) L. J. Ferrari, Thesis, Newark College of Engineering, 1955. Personal communication, Dr. D. F. Hinkley of Merck and Co.

(4) The author is indebted to Dr. D. F. Hinkley of Merck and Co. for a supply of pure a and crude b.

(5) G. W. H. Cheeseman, I. M. Heilbron, E. R. H. Jones, F. Sondheimer and B. C. L. Weedon, *J. Chem. Soc.*, 2031 (1949).

(6) Ultraviolet and infrared data were obtained on ethanolic and carbon bisulfide solutions, respectively, unless otherwise specified

(7) O. Isler, A. Ronco, W. Guex, N. C. Hindley, W. Huber, K. Dialer and M. Kofler, *Helv. Chim. Acta*, **32**, 489 (1949).

(8) The absence of iodine-stable 9-*cis*-vitamin A in the isomerate proves the absence of this configuration in the original compound.

(9) Prepared by the potassium borohydride-reduction of neoretinene b (*cf.* J. M. Dieterle and C. D. Robeson, *Science*, **120**, 219 (1954).