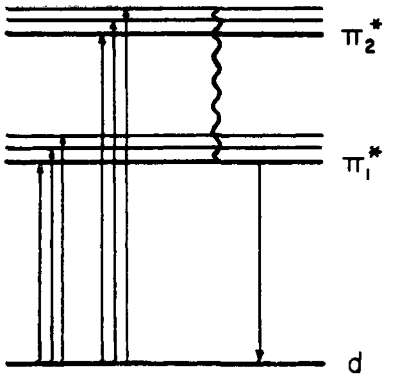

Fig. 2.—Energy scheme for the absorption and luminescence processes for tris-2,2′-bipyridineruthenium(II) chloride.

citation of fluorescence by any of the three absorption bands results in the same characteristic luminescence. Thus we have shown internal conversion from the higher excited level in the ultraviolet to the lowest excited level yielding fluorescence.

We wish to thank the National Science Foundation for its support of this work.

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RECEIVED MARCH 18, 1959

OPTICAL ROTATORY DISPERSION STUDIES. XXXI.[1] ANOMALOUS DISPERSION CURVES OF DITHIOCARBAMATES OF α-AMINO ACIDS AND PEPTIDES. STEREOCHEMICAL CORRELATIONS BETWEEN α-HYDROXY AND α-AMINO ACIDS[2]

Sir:

Anomalous Cotton effect curves recently have found extensive applications[3a] in many areas of organic chemistry. Unfortunately, most α-amino acids[3b] and their higher polymers[3c] exhibit only plain dispersion curves[4] over the presently accessible spectral range. We have now discovered that N-dithiocarbalkoxy α-amino acids (I) (dithiocarbamate derivatives) are almost uniquely suited for this purpose and that their use may represent an important advance in α-amino acid or peptide methodology. The preparation[5] of such derivatives is simple and often lends itself to work on a micro-scale.[6]

The long wave length absorption band near 330 mμ of such dithiocarbamates (I) is strongly optically active and leads to Cotton effect curves[4] (Fig. 1). *The sign of the Cotton effect of the dithiocarbamate will indicate the absolute configuration of the α-asymmetric center and all α-amino acids of the* L-*series, which have so far been examined, show a positive Cotton effect, the reverse being true of members of the* D-*series.* This has been tested with the dithiocarbamates of alanine, valine, phenylglycine, isoleucine, alloisoleucine, proline, aspartic acid, glutamic acid and cystine. The nearly identical positive Cotton effect curves of L-isoleucine and L-alloisoleucine demonstrate the unimportance of the β-asymmetric center in this type of analysis. Similarly, N-dithiocarbomethoxy L-alanyl-L-alanine and N-dithiocarbomethoxy L-alanyl-D-alanine exhibit positive Cotton effect curves of virtually identical amplitude. This suggests that the technique may be employed to determine the configuration of the α-asymmetric center of a terminal amino acid in a peptide chain, which may be of importance in antibiotic chemistry.

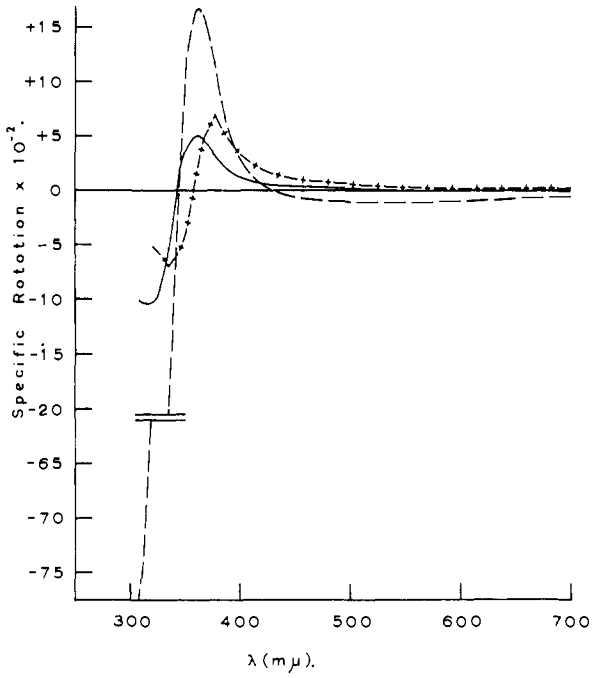
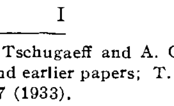


Fig. 1.—Optical rotatory dispersion curves (methanol solution) of N-dithiocarbomethoxy-L-(+)-valine, ———; N,N′-bis-dithiocarbethoxy-L-(−)-cystine, --------; and L-(−)-malic acid ethyl dithiocarbonate, —+—+—+.

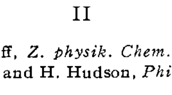
The "optical activity" of the dithiocarbamate is so strong as to overcome the structural factors which often confuse stereochemical correlations of the parent acid based on $[\alpha]_D$ (*e.g.*, L-(+)-alanine *vs.* L-(−)-cystine possessing dispersion curves of opposite sign[3b] while their dithiocarbamates exhibit positive Cotton effect curves).

Xanthate derivatives of certain alcohols have been shown[7] to give anomalous dispersion curves and we have now examined such derivatives (II) of α-hydroxy acids.



$$\mathrm{R{-}CH(NH{-}C({=}S){-}SR'){-}CO_2H}$$

I



$$\mathrm{R{-}CH(O{-}C({=}S){-}SR'){-}CO_2H}$$

II

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[1] Paper XXX, C. Djerassi, N. Finch and R. Mauli, THIS JOURNAL, **81**, 4997 (1959).

[2] Supported by grant No. CY-2919 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

[3] (a) C. Djerassi, "Optical Rotatory Dispersion: Applications to Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., Chapters 4–14; (b) Sec. 15-4 by J. A. Schellman in ref. 3a; (c) Chapter 17 by E. R. Blout in ref. 3a.

[4] For nomenclature see C. Djerassi and W. Klyne, *Proc. Chem. Soc.*, 55 (1957).

[5] See A. Fredga, *Svensk Kem. Tid.*, **53**, 221 (1941); **54**, 26 (1942).

[6] *E.g.*, 5.8 mg. of L-(−)-proline afforded 2.2 mg. of recrystallized dithiocarbamate.

[7] L. Tschugaeff and A. Ogorodnikoff, *Z. physik. Chem.*, **85**, 481 (1913), and earlier papers; T. M. Lowry and H. Hudson, *Phil. Trans.*, **A232**, 117 (1933).

In a few instances,[5] configurational relationships between α-amino and α-hydroxy acids have been established by the quasi-racemate method of derivatives of type I and II. We have now found that possibly the simplest manner for establishing such a relationship can be a comparison of the rotatory dispersion curves of a N-dithiocarbalkoxy α-amino acid (dithiocarbamate of α-amino acid) (I) and an α-hydroxy acid alkyl dithiocarbonate (xanthate of α-hydroxy acid) (II). Here, again, the confusion caused by opposite signs of $[\alpha]_D$ values of the parent substance is completely eliminated as was exemplified (see also Fig. 1) by the positive Cotton effect curves of the ethyl dithiocarbonates of L-(+)-lactic acid, L-(−)-malic acid and L-(+)-mandelic acid.

Details and further applications of this rotatory dispersion approach will be reported in our full papers.

(8) Postdoctorate research fellow (1958–1959) on leave from the University of Uppsala.

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RECEIVED AUGUST 17, 1959

PURIFICATION OF PARATHYROID HORMONE BY USE OF COUNTERCURRENT DISTRIBUTION

Sir:

The parathyroid hormone extracted from bovine glands with 70% phenol has been purified by countercurrent distribution in two solvent systems.

Crude gland powders were extracted with 70% aqueous phenol by a modification of the method of Aurbach.[1] After precipitation of the hormonal activity with ether, the active material was dissolved in 30% acetic acid. Precipitation of inactive protein was induced by the addition of 35% sodium chloride to a final salt concentration of 7.5%. The active material was then brought out of solution by precipitation with trichloroacetic acid at a final concentration of 7.5%. This TCA precipitate was dissolved in 0.1 *M* acetic acid, passed through a column of Dowex 2×8 acetate and then lyophilized. The resulting powder had a biological activity of 150–200 units/mg. dry weight when assayed for its calcium-mobilizing activity in parathyroidectomized rats.[2] This powder was further purified by use of countercurrent distribution. Two solvent systems were employed.

In a system of 6 parts of 6% acetic acid–1.0% NaCl and 4 parts of a 1:1 mixture of 1-butanol and 1-propanol (system 1) the material distributed as shown in Fig. 1. The substance having a *K* of 0.88 was the only hormonally active material and was found to have a potency of 2000–2500 units/mg.

The other system (system 2) was composed of 12 parts 0.1% acetic acid, 5 parts 1-butanol and 3.5 parts pyridine. After 200 transfers in this system the hormonal activity distributed with a *K* of 0.22. This was recovered, and rerun in the same system for 471 transfers. The pattern shown in Fig. 2 was obtained. Material from tubes 73–93 was recovered and found to have an activity of 2500–3000 units/mg. Because there is an error of approximately ± 25% with this assay procedure, the biological activities of the two preparations were not significantly different.

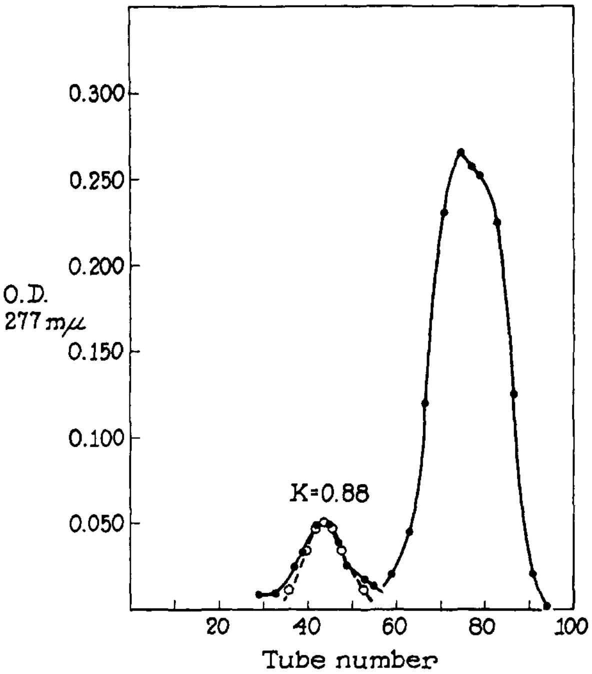

Fig. 1.—Countercurrent distribution pattern of parathyroid hormone after 100 transfers in system 1. The absorption at D_{277} ●—● and the theoretical distribution O—O are plotted.

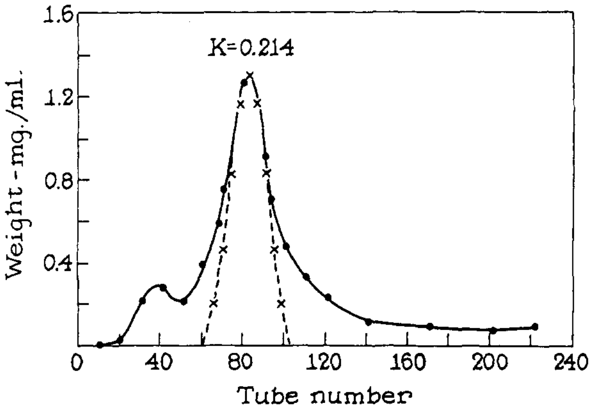

Fig. 2.—Countercurrent distribution pattern of parathyroid hormone after 471 transfers in system 2. The weight recovered ●—● and theoretical distribution X—X are plotted.

When the active substance from the pyridine system was distributed in system 1, it appeared as a single peak with a *K* of 0.86. The molecular weight estimated by both ultracentrifugation and dialysis was 7000 ± 1500. In both studies the material behaved as a single component.

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NEW YORK 21, N. Y. LYMAN C. CRAIG

RECEIVED JULY 29, 1959

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(1) G. D. Aurbach, *Arch. Biochem. Biophys.*, **80**, 466 (1959).

(2) P. L. Munson, *Ann. New York Acad. Sci.*, **60**, 776 (1955).