[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY OF STANFORD UNIVERSITY AND THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Optical Rotatory Dispersion Studies. LI.[1] Absolute Configurational Assignments of α-Amino Acids and Peptides through Anomalous Rotatory Dispersion of *N*-Phthaloyl Derivatives[2]

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Received May 15, 1961

The rotatory dispersion curves of a series of *N*-phthaloyl-α-amino acids, as well as their esters and amides, have been measured. While some difficulty is encountered in determining whether the extrema of the anomalous rotatory dispersion curves are necessarily involved in a Cotton effect associated with the first phthalimido absorption band, the sign of the dispersion curve can be related to the configuration of the α-asymmetric center. Attention is called to the spurious rotations which may be encountered occasionally with low rotating substances near their region of maximal absorption.

In a recent investigation[4] of the rotatory dispersion behavior of *N*-nitroso-*N*-methylamides of optically active acids, there were examined several *N*-nitroso derivatives of *N*-phthaloyl-α-amino acid *N*-methylamides. It was noticed immediately that their rotatory dispersion curves differed considerably from those of other *N*-nitroso-*N*-methylamides thus pointing towards a contribution from the *N*-phthaloyl grouping. As *N*-phthaloyl-α-amino-acids are used extensively in peptide synthesis,[5] it appeared desirable to study in some detail the rotatory dispersion behavior of such derivatives.

The ultraviolet absorption spectra of *N*-phthaloyl-α-amino acids are characterized by an intense maximum (log ε *ca.* 4.6) near 220 mμ and a weaker one (log ε *ca.* 3.3) at 292 mμ.[6] The latter occurs within the range of presently available spectropolarimeters[7] and it seemed appropriate to determine experimentally whether this band is optically active.[8]

Our experimental results are collected in Table I, and a few typical rotatory dispersion curves are reproduced in Fig. 1. Inspection of this experimental material reveals that while in several instances it is not possible to determine with certainty whether the 292 mμ absorption band is optically active, the over-all sign of the anomalous rotatory dispersion curve can apparently be used for stereochemical assignment. It will be noted that *N*-phthaloyl derivatives of L-α-amino acids exhibit

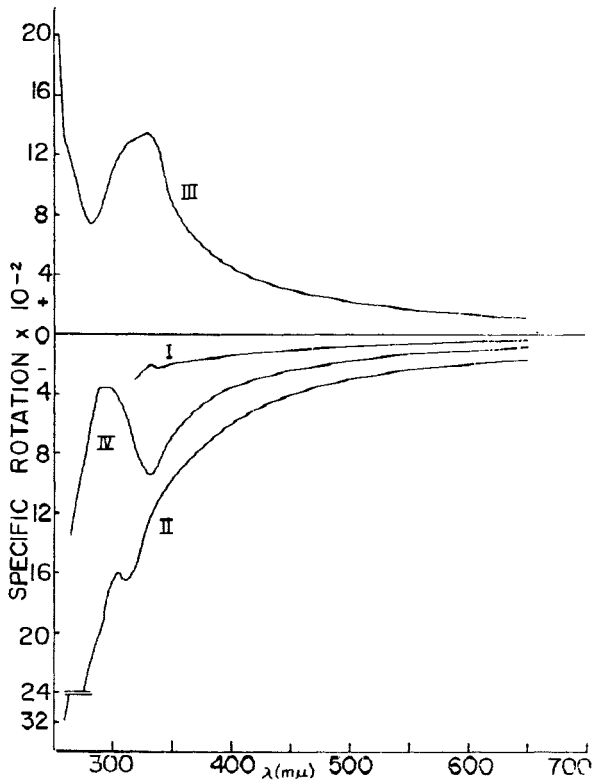

Fig. 1. Optical rotatory dispersion curves (methanol solution) of *N*-phthaloyl-L-valine (I), *N*-phthaloyl-L-phenylalanine (II), *N*-phthaloyl-D-phenylalanyl-L-proline methyl ester (III), and *N*-phthaloyl-L-alanyl-L-proline (IV)

(1) Paper L, J. Allinger, N. L. Allinger, L. E. Geller, and C. Djerassi, *J. Org. Chem.*, 26, 3521 (1961).

(2) Supported by the National Cancer Institute (grant No. CRTY-5061), National Institutes of Health, U. S. Public Health Service and by the National Science Foundation.

(3) National Institutes of Health Postdoctorate Research Fellow at Stanford University.

(4) C. Djerassi, E. Lund, E. Bunnenberg, and B. Sjöberg, *J. Am. Chem. Soc.*, 83, 2307 (1961).

(5) For pertinent references see J. C. Sheehan and V. S. Frank, *J. Am. Chem. Soc.*, 71, 1856 (1949); F. E. King and D. A. A. Kidd, *J. Chem. Soc.*, 3315 (1949); M. Goodman and G. W. Kenner in *Advances in Protein Chemistry*, Vol. XII, C. B. Anfinsen, Jr., M. L. Anson, K. Bailey, and J. T. Edsall, Editor, Academic Press, New York, 1957, p. 465.

(6) The following amino acid derivatives listed in Table I behaved differently: phthaloyl-L-histidine ($\lambda_{max}^{CH_3OH}$ 275 mμ), phthaloyl-*O*-acetyl-L-seryl-L-phenylalanine methyl ester (broad maximum centered at 280 mμ) and phthaloyl-L-threonyl-L-phenylalanine methyl ester (broad maximum centered at 280 mμ).

(7) C. Djerassi, *Optical Rotatory Dispersion: Applications to Organic Chemistry*, McGraw-Hill, New York, 1960, Chapter 3.

(8) During the course of our investigation there appeared a communication by J. H. Brewster and S. F. Osman, *J. Am. Chem. Soc.*, 82, 5754 (1960) in which they report a Cotton effect for phthalimides of optically active *p*-substituted α-phenylethylamines.

TABLE I

N-Phthaloyl Derivative of	Optical Rotary Dispersion		
	First extremum	Second extremum	Last reliable measurement and concentration
L-Valine[a,c]	$[\alpha]_{340}$ −228°	$[\alpha]_{333}$ −210° (see Fig. 1)	$[\alpha]_{318}$ − 316° (0.10)
L-Histidine[a,d]	$[\alpha]_{335}$ − 725°	—	$[\alpha]_{310}$ − 100° (0.03)
L-Threonine[a,e]	$[\alpha]_{335}$ − 155°	$[\alpha]_{332}$ − 50°	$[\alpha]_{315}$ − 416° (0.08)
L-Tyrosine[a,f]	$[\alpha]_{304}$ − 1580°	—	$[\alpha]_{300}$ − 1300° (0.03)
L-Glutamic acid[a,g]	$[\alpha]_{333}$ − 213°	—	$[\alpha]_{320}$ − 166° (0.105)
D-Phenylalanine[a,h] (for L-antipode see Fig. 1)	$[\alpha]_{309.5}$ + 1550° (infl.)	$[\alpha]_{290}$ + 1280°	$[\alpha]_{255}$ + 3200° (0.02)
L-Phenylalanine-*N*-methylamide[b,i]	$[\alpha]_{322}$ − 1245° and $[\alpha]_{320}$ − 1250°	$[\alpha]_{280}$ − 970°	$[\alpha]_{250}$ − 2425° (0.02)
L-Alanine-*N*-methylamide[b,j]	$[\alpha]_{325}$ − 125°	—	$[\alpha]_{323}$ − 32° (0.207)
L-Leucine-*N*-methylamide[a,k]	$[\alpha]_{343}$ − 109°	$[\alpha]_{295}$ + 69° (infl.)	$[\alpha]_{312}$ + 300° (0.10)
L-Alanyl-L-proline[a,l]	$[\alpha]_{330}$ − 937°	$[\alpha]_{295}$ − 375°	$[\alpha]_{265}$ − 1360° (0.03)
D-Phenylalanyl-L-proline methyl ester[a,m]	$[\alpha]_{332}$ + 1385°	$[\alpha]_{280}$ + 728°	$[\alpha]_{260}$ + 1360° (0.02)
L-Phenylalanylglycine[a,h]	$[\alpha]_{313}$ − 1057°	—	$[\alpha]_{305}$ − 925° (0.06)
L-Phenylalanylglycine ethyl ester[a,h]	$[\alpha]_{319}$ − 1078°	$[\alpha]_{300}$ − 865°	$[\alpha]_{260}$ − 1490° (0.021)
L-Threonyl-L-phenylalanine methyl ester[a,e]	$[\alpha]_{320}$ − 28°	—	$[\alpha]_{310}$ − 18° (0.113)
O-Acetyl-L-seryl-L-phenylalanine methyl ester[a,e]	$[\alpha]_{325}$ − 227°	—	$[\alpha]_{295}$ + 70° (0.102)
L-Valyl-γ-tosyl-L-ornithine ethyl ester[a,m]	$[\alpha]_{338}$ − 162°	—	$[\alpha]_{305}$ 0° (0.105)

[a] In methanol. [b] In dioxane. [c] M. Fling, F. N. Minard and S. W. Fox, *J. Am. Chem. Soc.*, **69**, 2466 (1947). [d] B. Helferich and H. Boshagen, *Chem. Ber.*, **92**, 2813 (1959). [e] J. C. Sheehan, M. Goodman and G. P. Hess, *J. Am. Chem. Soc.*, **78**, 1367 (1956). [f] S. Kanao, *J. Pharm. Soc. Japan*, **70**, 155 (1950); *Chem. Abstr.*, **44**, 5810 (1950). [g] J. H. Billman and W. F. Harting, *J. Am. Chem. Soc.*, **70**, 1473 (1948). [h] J. C. Sheehan, D. W. Chapman, and R. W. Roth, *J. Am. Chem. Soc.*, **74**, 3822 (1952). [i] M.p. 195–197°, $[\alpha]_D$ −133° (*c*, 5.2 in chloroform). *Anal.* Calcd. for $C_{18}H_{16}N_2O_3$: C, 70.11; H, 5.23; N, 9.09; O, 15.57. Found: C, 69.96; H, 5.20; N, 9.28; O, 15.81. [j] M.p. 160° dec., $[\alpha]_D$ −10° (*c*, 5.3 in chloroform). *Anal.* Calcd. for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.06. Found: C, 62.02; N, 5.11; N, 12.34. [k] M.p. 136–137°, $[\alpha]_D$ −31° (*c*, 5.16 in chloroform). *Anal.* Calcd. for $C_{15}H_{18}N_2O_3$: C, 65.67; H, 6.61; N, 10.21. Found: C, 65.34; H, 6.35; N, 10.23. [l] Unpublished work, J. C. Sheehan and G. P. Hess. [m] W. L. Richardson, Ph.D. thesis, M.I.T., 1954.

a negative dispersion curve,[9] while a positive one is associated with the corresponding member of the D series. This generalization holds for the free carboxylic acid, the *N*-methylamide and various peptide linkages. That only the asymmetric center involving the phthalimido function plays the governing role—thus making this approach useful for stereochemical assignments of terminal amino acids in a peptide sequence—is demonstrated in Fig. 1 with the rotatory dispersion curve of phthaloyl-D-phenylalanyl-L-proline methyl ester (III). While L-proline itself shows[10] a strong negative rotatory dispersion, the above phthaloyl peptide exhibits a strong positive curve as is the case (see Table I) with phthaloyl-D-phenylalanine itself.

Some comments on the experimental difficulties encountered in this work appear appropriate, especially as they are often not appreciated and may apply in part to some recently reported rotatory dispersion investigations.[8,11] The problem to which we are referring is the occasional appearance of spurious rotatory dispersion extrema near the region of maximal absorption (usually in the ultraviolet near the final wave length range of the instrument) of substances that do not possess very large rotations in that region. The combination of high dilution, relatively wide slit width, low rotation, and relatively high absorption can produce apparent Cotton effects, which are caused by stray light. We have occasionally encountered these even with the automatically recording Rudolph spectropolarimeter[12] equipped with improved polarizer and analyzer prisms. One index is the apparent "wandering" of this extremum upon altering the concentration, but the best check is to examine the "rotatory dispersion" of the corresponding optically inactive analog.

Turning to the specific case at hand, we have examined phthaloyl-D,L-alanine and found that the specific rotation is 0° ± 2° to about 300 mμ but that as one proceeds through the region of absorption, false rotations up to 200° (correspond-

(9) This does not necessarily mean that this represents a negative Cotton effect. For instance, the irregularities in the dispersion curve (Fig. 1) of phthaloyl-L-phenylalanine (II) do occur within the region of the phthalimide absorption band, but it is difficult to decide from an inspection of this curve whether this represents a true Cotton effect and what sign such a Cotton effect should be attributed.

(10) See section 15-4 (by J. A. Schellman) in ref. 7.

(11) G. G. Lyle, *J. Org. Chem.*, **25**, 1779 (1960).

(12) H. Rudolph and R. Bruce, *J. Opt. Soc., Am.*, **49**, 1127 (1959). Continuous monitoring of the dynode voltage applied to the photomultiplier (RCA 7200) is helpful in judging the reliability of the observed rotations. We have considered a dynode voltage of 600 volts (photomultiplier at ambient temperature) as indicative of the limit of reliability.

ing to 0.01° in actual rotation with a concentration of $c = 0.02$) may be encountered. It is for this reason that we have listed in Table I not only the positions of the extrema[13] but also what we consider to be the last reliable measurement. It will be noted that the position of this last significant measurement covers quite a range, farther penetration into the ultraviolet being associated with higher rotation. Attention should be drawn to the threonine derivatives, which possess rather low rotations even in the experimentally significant region. As has been noted by Shellman[10] for threonine itself, this is probably due to the compensating effect of the second asymmetric center bearing the hydroxyl group.

We have already shown earlier that the sign of the Cotton effect of certain α-amino acid derivatives such as *N*-dithiocarbalkoxy[14] and *N*-thionocarbalkoxy[15] analogs can be used for purposes of attributing absolute configurations to α-amino acids or terminal amino acids in a peptide sequence. While these derivatives show true Cotton effects—in contrast to the possible ambiguities discussed above for phthalimido α-amino acids—the sign of the latter's dispersion curve can apparently be used equally effectively for stereochemical assignments. The use of *N*-phthaloyl derivatives has the advantage of employing intermediates which are of synthetic utility[5] rather than involving derivatives[14] which are prepared specifically for rotatory dispersion measurements because of their desirable spectral properties.

(13) In our opinion, it is rather difficult to decide at this point whether some of the extrema listed in Table I are in fact extrema of a Cotton effect or rather turning points where a Cotton effect of one sign overcomes the strong "background" rotation (for definition see p. 16 of ref. 7) of opposite sign. Such turning points are usually flat and rounded, yet the first extremum of III in Fig. 1, while showing such a shape, also appears to consist of fine structure and may, in fact, contain the first extremum of a positive Cotton effect.

(14) B. Sjöberg, A. Fredga, and C. Djerassi, *J. Am. Chem. Soc.*, 81, 5002 (1959).

EXPERIMENTAL[16]

All optical rotatory dispersion measurements were conducted in methanol or dioxane solution with 0.5- or 0.1-dcm. cells and concentrations in the range *c*, 0.1 (700–310 mμ)–0.02 (below 310 mμ) using a Rudolph automatically recording spectropolarimeter.[7,12] The results are summarized in Table I.

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(15) C. Djerassi, K. Undheim, R. C. Sheppard, W. G. Terry, and B. Sjöberg, *Acta. Chem. Scand., in press.*

(16) We are greatly indebted to Mrs. Ruth Records for technical assistance.

[CONTRIBUTION FROM THE ORGANIC CHEMISTRY LABORATORY, THE INSTITUTE OF PHYSICAL AND CHEMICAL RESEARCH]

Syntheses of DL-Isoleucine Based on the Darapsky and the Hofmann Degradations

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Received September 6, 1960

DL-Isoleucine was synthesized from ethyl 2-cyano-3-methyl-2-pentenoate *via* ethyl 2-cyano-3-methylvalerate in two ways—Darapsky's method (—$COOC_2H_5$ ⟶ —NH_2) and the Hofmann method (—CN ⟶ —$CONH_2$ ⟶ —NH_2). More isoleucine was found in the crude product made by Darapsky's method than by the Hofmann method.

Further, a similar experiment *via* ethyl 2-cyano-3-methylvalerate which was prepared from ethyl cyanoacetate and *sec*-butyl bromide was carried out. In this case, the crude product was richer in isoleucine by the Hofmann method than by Darapsky's.

Numerous syntheses of DL-isoleucine give alloisoleucine simultaneously. Doyle *et al.*[1] have prepared this amino acid by some classical and newer methods, and have conducted precise bioassays for isoleucine content in the crude products. It seems from their results that the stereospecific synthesis of DL-isoleucine was not fully accomplished.

In the present experiment, ethyl 2-cyano-3-methyl-2-pentenoate (I), obtained by condensing methyl ethyl ketone with ethyl cyanoacetate, was a starting material. If I is a mixture of *cis* and *trans* isomers (Ia and Ib), ethyl 2-cyano-3-methylvalerate (II), given by catalytic hydrogenation of I, should consist of two racemic diastereoisomers (IIa and IIb). There are two methods to prepare an α-amino acid in this case. The first is to make the required compound by Darapsky's method[2] (a modified Curtius's reaction) which converts the ethoxycarbonyl group into an amino group *via* an hydrazinocarbonyl group and the cyano group

(1) F. P. Doyle, D. O. Holland, W. Marflitt, J. H. C. Nayler, and (Miss) C. M. O'Connor, *J. Chem. Soc.*, 1719 1955).

(2) A. Darapsky and D. Hillers, *J. prakt. Chem.*, 92, 297 (1915); A. Darapsky, *J. prakt. Chem.*, 146, 250 (1936).