A TEST TUBE REACTION FOR THE DETERMINATION OF THE ABSOLUTE CONFIGURATION OF α-AMINO ACIDS

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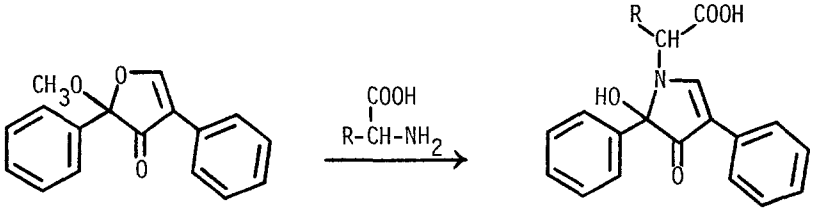
SUMMARY

2-Methoxy-2,4-diphenyl-3(2H)-furanone (MDPF) reacts readily with α-amino acids to form pyrrolinone-type chromophores with long wavelength absorption maxima at 370-390 nm. Measurement of the chiroptical properties of such reaction mixtures, without isolation of products, enables one to determine the absolute configuration of α-amino acids in situ.

INTRODUCTION

We have recently described the application of 2-methoxy-2,4-diphenyl-3(2H)-furanone (MDPF) I as a new chromophoric reagent for the determination of the absolute configuration of α-amino acids (1). A number of chromophores II were synthesized with various α-amino acids and their ORD and CD spectra were recorded in



I II

ethanol. Without exception, the L-amino acid derivatives showed first positive Cotton effects centered at ca 380 nm and second negative Cotton effects centered at ca 325 nm. Within the experimental error, the ORD and CD curves of the D-amino acid condensation products were mirror images of those of L-configuration. The chromophoric reaction between MDPF and

an α-amino acid can be carried out in a test tube and CD spectra can be obtained from the resulting reaction mixtures without the isolation of the new derivatives II. This procedure provides a rapid and simple method for the determination of the absolute configuration of α-amino acids in solution (2).

A number of methods for the preparation of chromophoric derivatives of α-amino acids have been described in the literature (3,4,5), and some of their disadvantages have been noted (5,6). In some instances the sign of the Cotton effect of the derivative is not only the function of the configuration, but is also dependent upon the nature of the substituents of the α-carbon. Furthermore, derivatization is often accompanied by partial racemization or the reagent (chromophoric or chelating) may also react with the hydroxy or sulfhydryl groups (6,7).

EXPERIMENTAL

A. Reagents

MDPF was obtained from Hoffmann-La Roche Inc., Nutley, New Jersey. Methanol (AR-grade) and dioxane (histological grade) were purchased from Fischer Scientific Co., Fair Lawn, New Jersey, and the amino acids from Fox Chemical Co., Los Angeles, California. Triethylamine was obtained from Eastman Kodak Co., Rochester, New York.

B. Method

General procedure: 0.5 ml of a 0.02 M (concentration may range between 0.1 and 0.002 M) of an α-amino acid in 50% methanol/water (or in 50% dioxane/water) is neutralized with 1 equivalent of triethylamine (0.025 ml of a 0.4 molar solution in methanol or dioxane). Then 0.475 ml of a 0.0421 molar (2 equivalents) solution of MDPF in methanol (or in dioxane) is added under stirring. The reaction mixture is heated at 50°C for 5 minutes, cooled, and transferred into a 0.01 cm cell (total volume of 0.5 ml required) and the CD spectra are recorded on a Durrum-JASCO Spectropolarimeter, Model ORD/CD/UV-5 between 450 and 270 nm. The spectra are difficult to obtain below 270 nm because of the high absorption of the reagent.

On a microscale, useful CD spectra (signal to noise ratio higher than 10) were obtained with 0.01 mg of amino acids. In order to avoid working with dilute solutions, where the reaction is slow or incomplete, 0.01 mg of an amino acid was dissolved in 0.01 ml 50% methanol/water and reacted in a test tube with 2 equivalents of MDPF in 0.01 ml methanol containing 1 equivalent of triethylamine. After heating at 50°C for 5 minutes, 5 ml

of methanol were added and the CD spectra recorded in 5 cm cells (total volume of 2.5 ml needed).

RESULTS AND DISCUSSION

As previously described, α-amino acids react with MDPF I to form N-substituted-3,5-diphenyl-5-hydroxy-2-pyrrolin-4-ones II. These derivatives exhibit multiple Cotton effects between 400 and 200 nm. In the case of L-amino acids, the first characteristic Cotton effect at ca 385 nm is positive and the second one at ca 325 nm is negative. There are three to four additional Cotton effects between 300 and 200 nm, depending on the character of the amino acid.

For many purposes, the reaction between I and α-amino acids can be carried out in a fashion which obviates the need for the isolation of the

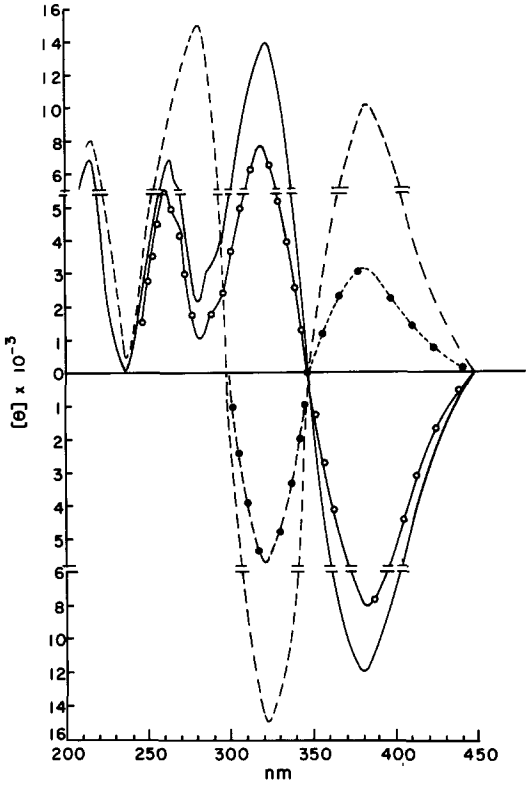


Fig. 1 CD spectra of synthesized (isolated) chromophores of D-phenylalanine (—) and L-leucine (---) and of the *in situ* reaction products of D-phenylalanine (O-O) and L-leucine (•--•) with MPDF.

Table I

First and Second Cotton Effects in CD Spectra of Reaction Products of α-Amino Acids with MDPF in Situ

Amino Acid	Predicted Sign 1st	Predicted Sign 2nd	Experimental in Situ 1st λnm	Experimental in Situ 1st $[\theta]\times10^{-3}$	Experimental in Situ 2nd λnm	Experimental in Situ 2nd $[\theta]\times10^{-3}$	Standards(1) 1st λnm	Standards(1) 1st $[\theta]\times10^{-3}$	Standards(1) 2nd λnm	Standards(1) 2nd $[\theta]\times10^{-3}$	% of Standard[c] 1st	% of Standard[c] 2nd
L-Alanine	+	-	385	- 1.10	320	- 4.70	384	+ 0.78	327	- 8.41	-	55.8
D-Alanine	-	+	385	+ 1.20	322	+ 4.60	385	- 1.61	325	+ 7.38	-	62.4
L-Leucine	+	-	384	+ 3.10	322	+ 5.70	385	+10.12	324	-15.20	30.6	37.2
D-Leucine	-	+	386	- 3.60	322	+ 5.70	385	- 9.89	323	+14.05	36.4	40.5
L-Isoleucine	+	-	388	+ 2.60	318	- 2.55	385	+ 8.00	323	- 7.60	32.5	34.5
D-Isoleucine	-	+	389	- 2.70	320	+ 2.30	385	- 7.65	323	+ 6.52	35.3	35.4
L-Threonine	+	-	376	+ 2.50	318	- 3.37	381	+ 8.15	322	-11.22	31.2	30.0
D-Threonine	-	+	380	- 2.94	319	+ 4.19	380	-11.16	323	+13.82	26.3	30.4
L-Methionine	+	-	382	+ 4.75	318	- 3.70	385	+11.20	325	-12.26	42.4	30.3
D-Methionine	-	+	386	- 3.60	319	+ 3.80	387	-10.62	325	+10.15	33.9	37.5
D-Glutamic Acid	-	+	375	- 1.25	320	+ 2.50	376	- 2.78	320	+ 5.08	45.0	49.2
L-Glutamine	+	-	380	+ 2.00	321	- 2.20	383	+ 5.82	325	- 6.60	34.4	34.4
L-Phenylalamine	+	-	380	+ 7.80	321	-74.00	382	+11.48	323	-13.83	67.8	54.6
D-Phenylalamine	-	+	385	- 8.00	320	+ 7.60	382	-12.00	325	+13.82	66.7	55.0
D-Phenylglycine	+	-	385	- 5.60	325	+ 6.00	385	-16.14	322	+16.14	34.7	37.2
L-Tryptophan	+	-	383	+ 8.40	315	- 8.60	384	+12.49	317	-19.40	67.3	44.3
D-Tryptophan	-	+	389	- 9.40	320	+ 8.10	383	-13.90	318	+19.02	67.6	42.6
L-Arginine.HCl[a]	+	-	384	+ 6.50	315	- 7.30						
L-Histidine.HCl[a]	+	-	376	+12.50	316	- 8.80						
D-Histidine.HCl[a]	-	+	385	-12.60	323	+ 9.45						
L-Dopa[a]	+	-	386	+ 7.80	323	- 4.10						
L-CH_3OCH=CH-CH(NH$_2$)-COOH[a,b]	+	-	387	+ 1.65	322	- 3.52						

(a) No synthesized standards available; (b) Obtained from Dr. J. Scannell, Hoffmann-La Roche Inc.
(c) Parameters to increase the yield are under investigation (variation of solvents, temperature and concentration of triethylamine and MDPF etc.).

chromophores II, and allows one to obtain useful chiroptical spectra with the reaction mixture itself.

CD data obtained with such reaction mixtures are summarized in Table I. The specific spectra obtained with the reaction mixtures of D-phenylalanine and L-leucine are depicted in Figure 1.

The first two characteristic Cotton effects of the chromophore II produced in situ are easily accessible for configurational or analytical purposes. In the presence of excess reagent (UV max in methanol at 241 nm, ε = 18800 and at 308 nm, ε = 3500), the cutoff wavelength (under standard conditions) is approximately 260-270 nm and the third Cotton effect is, in most cases, difficult to measure. Only in the case of phenylalanine could the CD spectrum be recorded between 450 and 220 nm (Figure 1).

As seen from the Table, the intensities of the first two analytically accessible Cotton effects of the reaction mixtures are ca 30-70% of those of the isolated standards (1). Since the Cotton effects are strong, these yields are sufficient for in situ determination of the absolute configuration of α-amino acids in solution. On a microscale, the color reaction was successfully carried out with as little as 0.01 mg of amino acids (8).

The signs of measured and predicted Cotton effects (based on those of the synthesized ones) are in agreement. The only exception observed is alanine, where the sign of the first Cotton effect in situ is opposite to that of the standard, but the sign of the second one at 325 nm remains as expected. The reason for this could be due to a solvent effect, or more likely, to solvent induced mutarotation resulting from epimerization of the carbinolamine function. Therefore, it is advisable to measure the first and the second Cotton effect.

ACKNOWLEDGEMENTS

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8. Only 1-2 micrograms of the synthesized chromophores (isolated) are needed for useful CD spectra.