CHIROPTICAL PROPERTIES OF FLUORESCAMINE CONDENSATION COMPOUNDS WITH α-AMINO ACIDS IN SITU.

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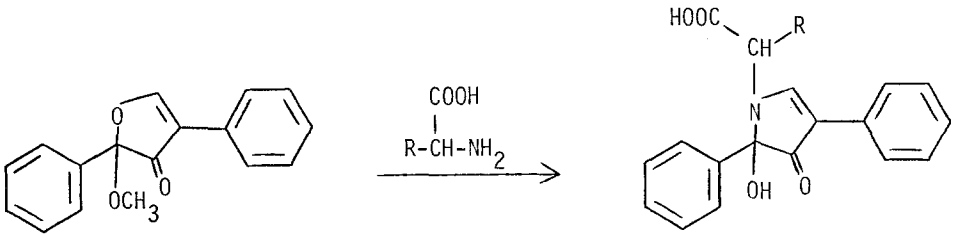
SUMMARY

Fluorescamine reacts efficiently with primary amino acids to form pyrrolinone type chromophores. A simple test tube procedure is described which allows in situ determination of absolute configuration of α-amino acids based on the chiroptical properties of these chromophoric derivatives.

INTRODUCTION

It has been reported (1) that 2-methoxy-2,4-diphenyl-3-(2H)-furanone (MDPF) I reacts with primary amines to form corresponding N-substituted 3,5-diphenyl-5-hydroxypyrrolin-4-one-type chromophores II. We have recently described the synthesis and the chiroptical properties of the chromophoric MDPF derivatives of a variety of D- and L-α-amino acids (2). It was found that the first Cotton effects in the CD spectra of the L-amino acid derivatives (at ca 380 nm) are always positive, while the second Cotton effects (at ca 325 nm) are negative. The ORD and CD spectra of the D-amino acid condensation products were mirror images of those of L-configuration. Subsequently, it was demonstrated that such chiroptical information can also be obtained directly from the reaction mixtures of amino acids and MDPF in situ (3), without prior isolation of the chromophoric derivatives.



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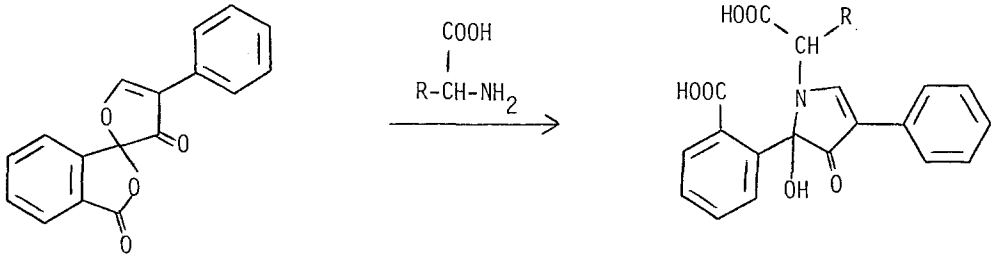

III IV

The related reagent fluorescamine, 4-phenylspiro [furan-2(3H), 1'-phthalan]-3,3'-dione III, which has been widely used for the fluorometric and colorimetric assay of primary amines (1,4,5,6) also forms chiroptically active chromophores IV with α-amino acids. This reaction is simple and fast and can be performed in test tubes. CD spectra can be obtained from the resulting mixtures without isolation of the chromophores IV. The advantages and disadvantages of the fluorescamine procedure in comparison with the MDPF methods are discussed.

EXPERIMENTAL

A. Reagents

Fluorescamine (Fluram®) was obtained from Hoffmann-La Roche Inc., Nutley, New Jersey, and histological grade dioxane from Fischer Scientific Co., Fair Lawn, New Jersey. The amino acids were purchased from Fox Chemical Company, Los Angeles, California. The phosphate or borate buffers (0.05M) were prepared accord

ing to Clark and Lubs (7) using AR-grade chemicals from Mallinckrodt Chemical Works, St. Louis, Mo.

B. Method

Two ml of a 0.004 M solution of fluorescamine in dioxane are rapidly added to 2 ml of a 0.002 M (concentration may range between 10^{-2} and 0.5×10^{-6} M) solution of an α-amino acid in phosphate or borate buffer pH 8-9 in a test tube. The reaction mixture is stirred for 15 sec. on a Vortex-type mixer, transferred into a 0.1 cm cell (or into a cell of different length, depending on the amino acid concentration) and the CD spectra are recorded on a Durrum-JASCO spectropolarimeter, Model ORD/CD/UV-5 between 450 and 270 nm (in the case of the D-phenylalanine and L-leucine, between 450 and 220 nm). The spectra are difficult to obtain below 270 nm because of the high absorption of the reagent, especially if its concentration is higher than 0.004 M.

RESULTS AND DISCUSSION

The pyrrolinone-type chromophores arising from the reaction of MDPF or fluorescamine with primary amino acids are similar and their absorption maxima are located in the 270-280 (ε=18000-20000) and 380-390 nm regions (ε=6000-7000). However, the reaction products of the primary amino acids with fluorescamine are only stable *in situ* (under the conditions which are optimal for their formation) and they decompose partially under conditions required for their isolation (e.g. neutralization).

For the reasons previously discussed (5), the amino acids are dissolved in pH 8-9 phosphate or borate buffers and reacted with fluorescamine in dioxane. Although the reactions are complete within one minute it is sometimes advisable to let the reaction mixtures stand for 10-20 minutes for optimum development of Cotton effects. When the amino acid concentration is in the range of 0.01-0.001 M, a twofold excess of fluorescamine is sufficient for the maximum chemical yield *in situ*, but lower amino acid concentrations require a 20-40 fold excess of the reagent (6).

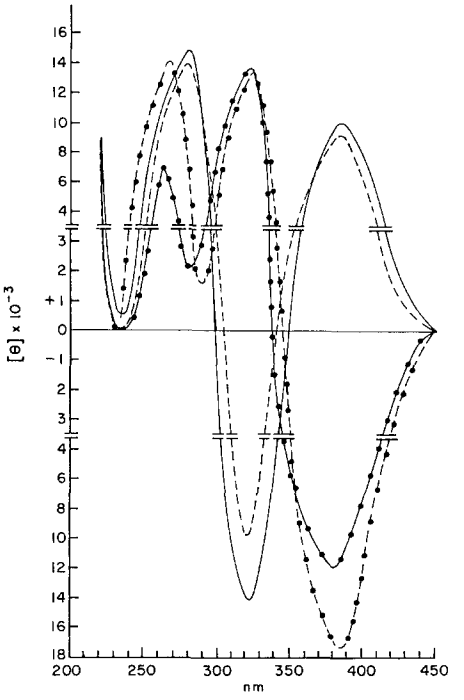

Fig. 1 CD spectra of synthesized (isolated) chromophoric derivatives of D-phenylalanine (●——●) and L-leucine (———) with MDPF in ethanol and of the in situ reaction mixtures of D-phenylalanine (● – ●) and L-leucine (— —) with fluorescamine.

The CD spectra of the reaction mixtures show 3-4 Cotton effects between 400 and 220 nm as shown in Fig. 1 in the case of D-phenylalanine and L-leucine, but only the first and second one (at 380 and 325 nm resp.) are readily accessible. For comparison, the CD spectra of the isolated chromophoric derivatives of D-pheny alanine and L-leucine with MDPF are also depicted in Fig. 1. A number of amino acids were reacted with fluorescamine and first and second Cotton effects of the reaction mixtures were recorded. The data which were obtained are summarized in Table I.

As with the MDPF derivatives, the first Cotton effects of the chromophores derived from L-amino acids and fluorescamine are positive and the second Cotton effects are negative. Within the experimental error, the CD curves of the D-amino acid-derived chromophores are mirror images of those of the L-amino acids. The

TABLE I

First and Second Cotton Effects in CD Spectra of Reaction Products of α-Amino Acids with Fluorescamine in Situ[a]

Amino Acid	Predicted Signs		Experimental in Situ			
	1st	2nd	1st		2nd	
			λnm	$[\theta]x10^{-3}$	λnm	$[\theta]x10^{-3}$
L-Alanine	+	-	380	- 1.70	324	- 4.05
D-Alanine	-	+	380	+ 1.80	323	+ 3.90
L-Leucine	+	-	385	+ 9.20	321	- 9.85
D-Leucine	-	+	385	- 9.45	322	+10.05
L-Isoleucine	+	-	389	- 5.55	319	- 7.40
D-Threonine	-	+	377	- 2.50	318	+ 5.80
L-Methionine	+	-	385	+ 7.95	320	- 5.30
D-Methionine	-	+	385	- 8.20	318	+ 5.25
L-Glutamic Acid[b]	+	-	379	+ 1.57	319	- 1.68
L-Arginine .HCl[b,c]	+	-	385	+ 1.42	325	- 4.63
L-Glutamine[b]	+	-	379	+ 1.50	324	- 1.10
L-Cysteine	+	-	396	+ 7.58	320	- 2.65
L-Histidine .HCl	+	-	385	+11.60	322	-10.85
D-Histidine .HCl	-	+	385	-11.60	322	+10.90
L-Dopa	+	-	387	+17.95	315(sh)	-11.95
L-Phenylalanine	+	-	385	+17.40	325	-13.57
D-Phenylalanine	-	+	385	-17.15	326	+13.25
L-Phenylglycine	+	-	386	+16.00	320	- 5.33
D-Phenylglycine	-	+	386	-15.40	319	+ 5.85
L-Tryptophan	+	-	386	+18.76	310(sh)	-15.40

(a) Final concentration 0.001M unless otherwise stated. (b) Final concentration 0.01M; parameters to increase the yield (variation of solvents, pH, concentration of fluorascamine etc.) are under investigation. (c) Chromophoric derivative quite unstable. (Sh) = Shoulder.

only exception to this rule is [as in the case with MDPF (3)] the L-alanine reaction product where the sign of the first Cotton effect is reversed, but the sign of the second one remains unchanged and can be safely used for configurational purposes. Therefore, it is advisable to measure the first and the second Cotton effect.

Under standard conditions, the fluorescamine reaction is more sensitive than the MDPF method. As little as 0.1-1.0 μg/ml of amino acids has been routinely

reacted with fluorescamine and useful CD spectra of the reaction mixtures were obtained.

The main advantage of this fluorescamine method is its simplicity. In this test tube procedure fluorescamine reacts more reproducibly with the amino acids than does MDPF and, therefore, the reaction mixtures obtained with the first reagent are more suitable for quantitative analysis. For the determination of absolute configuration of more complex amino acids it may be essential to isolate and characterize the chromophoric derivative. In such cases MDPF will be the reagent of choice (2). However, in many other situations the outlined simple test tube procedure with fluorescamine will suffice to furnish the desired information.

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