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Application of Fluorescamine XII.[1] Chiroptical Properties of Fluorescamine Condensation Compounds with Some Less Common α-Amino Acids

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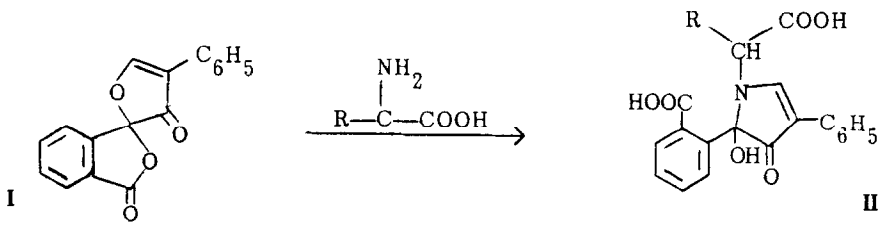
Summary

Fluorescamine (FLURAM®) reacts efficiently with less common chiral α-amino acids to form pyrrolinone-type chromophores. The characteristic Cotton effects at 324-300 and at 290-263 nm can be related to the absolute configuration of the parent α-amino acid without isolation of the products. These CD bands are negative and positive, respectively, for the derivatives of the L-α-amino acids.

Introduction

We have recently reported on the application of fluorescamine, 4-phenylspiro(furan-2(3H), 1'-phthalan)-3,3'-dione I as a chromophoric reagent for the determination of absolute configuration of primary α-amino acids (2), the NH_2-terminal amino acids of dipeptides (3) and amino acid esters (1). It is shown that in every case investigated so far, the sign of the second Cotton effect (around 325-300 nm) can be safely used for the determination of the absolute configuration of the α-amino acids, α-amino acid esters and the NH_2-terminal amino acids of dipeptides: it is negative for the chromophoric derivatives of L-configuration and positive for the derivatives of D-configuration (4). Because of the difference of chiroptical properties (reversal of the sign of the first Cotton effect in several cases) between the fluorescamine derivatives of α-amino acids, α-amino acid esters and dipeptides (1,2,3), it was of interest to investigate the chiroptical properties of less common α-amino acids.

Fluorescamine I reacts efficiently with less common α-amino acids (names and structures given in Table I) to form pyrrolinone-type chromophores II:



I II

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As in the previous cases, this reaction is simple and fast and can be performed in test tubes under mild conditions, and the CD spectra are obtained from the resulting mixtures without isolation of the chromophore II.

Experimental

A. Reagents

Some of the amino acids were purchased from Pierce Chemical Co., Aldrich Chemical Co. and Vega-Fox Biochemicals and were used without further purification (see Table I). Others were obtained from Dr. J. P. Scannell, Hoffmann-La Roche Inc. Histological grade dioxane was purchased from the Fischer Scientific Company and fluorescamine (FLURAM®) was obtained from Hoffmann-La Roche Inc. The phosphate buffer (0.05 M, pH 8-8.5) was prepared according to Clark and Lubs (5) using AR-grade chemicals from Mallinckrodt Chemical Works.

B. Method

Two ml of a 0.004 M solution of fluorescamine in dioxane is 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 amino acid in 0.05 M phosphate buffer, pH 8-8.5, in a test tube. The reaction mixture is stirred ca 20 sec. on a Vortex mixer, transferred to a 0.1 cm cell (or into a cell of different length, depending on the amino acid concentration and the wavelength range), and the CD spectra are recorded after one minute on a JASCO spectropolarimeter, Model J-20, between 450 and 240 nm. The spectra are difficult to obtain below 260 nm due to the unfavorable anisotropy factor $\Delta\varepsilon/\varepsilon$.

Results and Discussion

For reasons previously discussed (6), the amino acids are dissolved in phosphate buffer (pH 8-8.5) and reacted with fluorescamine in dioxane. When the amino acid concentration is in the range of 0.01-0.001 M, a two- to fourfold excess of fluorescamine is sufficient for analytical purposes, but lower amino acid concentrations ($\leq 10^{-4}$M) require a 20-40 fold excess of the reagent (7). Although under these conditions the formation of the pyrrolinone-type chromophore II is complete within one minute, it is sometimes advisable to let the reaction mixture stand for 10-20 minutes for the maximum chemical yield in situ. The chromophores are stable at least for 1-2 hours.

A number of less common L-α-amino acids (no D-enantiomers were available) were reacted with fluorescamine and the CD spectra were recorded between 450 and 240 nm. The position, sign and intensity of the Cotton effects are summarized in Table I. In Figure 1 the spectra of the reaction products of compounds 2, 3 and 4 with fluorescamine in situ are shown.

TABLE I

Cotton Effects in CD Spectra of Reaction Products of Less Common α-Amino Acids with Fluorescamine in Situ (i = inflection)

Amino Acid	1st				2nd		3rd Cotton Effect[a]	
	nm	$(\theta)x10^{-3}$	nm	$(\theta)x10^{-3}$	nm	$(\theta)x10^{-3}$	nm	$(\theta)x10^{-3}$
1. L-Allylglycine[b] (L-2-Amino-4-pentenoic acid) CH_2=CH-CH_2-CH(NH_2)COOH	389	+ 9.15	343(i)	+4.41	318	- 3.82	282	+16.45
2. L-2-Amino-4-(2-aminoethoxy)-butanoic acid[e] H_2N-CH_2-CH_2-O-CH_2-CH_2-CH(NH_2)COOH	398	+ 5.11	354	+0.72	319	- 4.07	281	+12.55
3. L-Trans-2-amino-4-(2-aminoethoxy)-3-butanoic acid H_2N-CH_2-CH_2-O-CH=CH-CH(NH_2)COOH[e]	395	+10.30	353(i)	+1.80	324	- 8.69	288	+11.55
4. S-2-(Aminoethyl)-L-cysteine · HCl H_2N-CH_2-CH_2-S-CH_2-CH(NH_2)COOH · HCl	400	+13.18	345	+2.80	319	- 2.65	290	+ 8.46
5. L-p-Aminophenylalanine[d] p-H_2N-C_6H_4-CH(NH_2)COOH	414	+23.00	365	+5.61	301	-30.06	270	+10.81
6. L-Citrolline[d] (L-2-Amido-5-ureidovaleric acid[d] H_2N-CO-NH-$(CH_2)_3$-CH(NH_2)COOH	392	+ 3.35	351	+2.68	318	- 5.95	279	+17.84
7. 2,4-Diaminobutyric acid · HBr[d] H_2N-CH_2-CH_2-CH(NH_2)COOH · HBr	404	+14.07	365	-2.50	319	- 5.38	280	+ 7.45
8. L-Methionine-DL-sulfoxime[c] CH_3-S(O)(=NH)-CH_2-CH_2-CH(NH_2)COOH	388	+ 4.56	345	+5.20	316	- 4.84	275	+14.42
9. L-Threo-β-hydroxyaspartic acid[c] HOOC-CH(OH)-CH(NH_2)COOH	390	- 2.45	348	+0.51	315	- 7.56	263	+ 7.65
10. L-Threo-β-methylglutamic acid[e] HOOC-CH_2-CH(CH_3)-CH(NH_2)COOH	390	+ 2.75	345(s)	+0.70	317	- 4.75	280	+10.20

a = (θ) are reported in reference to the *molar* concentrations of amino acids in the reaction mixture. (S) = shoulder. b = From Aldrich Chemical Co. c = From Pierce Chemical Co. d = From Vega-Fox Biochemicals. e = From Dr. J. P. Scannell, Hoffmann-La Roche Inc., Nutley, N.J.

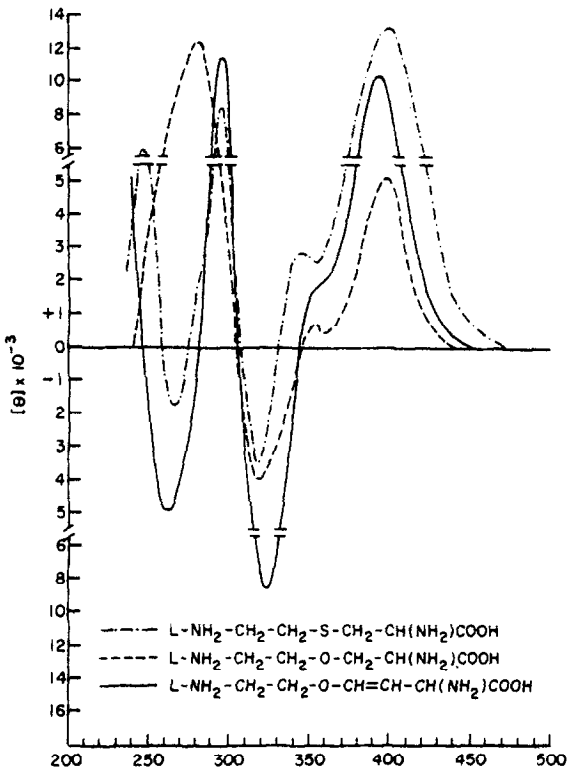

Fig. 1 CD spectra of the in situ reaction mixtures of compounds 2, 3 and 4 with fluorescamine in 0.05 M phosphate buffer pH 8/dioxane, 1:1, v./v.

The pyrrolinone-type chromophores II show three main characteristic Cotton effects between 404 and 260 nm. The sign of the first Cotton effects (at 414-388 nm) is positive. The only exception is the L-threo-β-hydroxyaspartic acid reaction product where the sign of the first Cotton effect is reversed. But in every case the sign of the second Cotton effect (at 324-301 nm) is always negative and can be safely used for the determination of the absolute configuration of less common amino acids. The sign of the third Cotton effect located at 290-263 nm is opposite to that of the second one. The sign pattern of the second and third Cotton effects (negative/positive) is similar to those of the fluorescamine derivatives of amino acids (2), amino acid esters (1) and N-terminal amino acids of dipeptides (3) of L-series. The importance and the reliability of the second and possibly third Cotton effect has been stressed in previous investigations (1,2,3).

The only difference in the CD spectral pattern of the less common L-α-amino acid derivatives when compared with those previously described (1,2,3), is the appearance of a weak Cotton effect (sometimes shown only as an inflection or a shoulder) in the 365-343 nm region. At present we have no explanation for this extra band (8).

The UV spectra of the pyrrolinone-type chromophores II (9) show maxima at 280-285 nm (Σ= 16000-18000) and at 385-390 nm (Σ=6000-7000) corresponding to the first and the third Cotton effects. The second CD band is observed in the area (324-301 nm) where the UV spectra of the pyrrolinone-type chromophore show minima. It is presumed that it represents the long wavelength portion of a couplet resulting from the exciton splitting between the 280-285 nm transition moment of the electron transfer band of the pyrrolinone chromophore and the recently described transition ($\varepsilon \approx 10^{-2}$, supposedly a singlet → triplet transition of the carbonyl group) of the carboxylic acids and esters in the vicinity of 275 nm (10). A pyrrolinone chirality rule has been proposed to explain the sign of the second CD band (1).

The main advantage of this fluorescamine method is its simplicity. Under standard conditions as little as 1 μg/ml of amino acids has been routinely reacted with fluorescamine, and useful CD spectra of the reaction mixtures were obtained.

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4. Recently, Kovacs, K. L., (1979) Biochem. Biophys. Res. Comm., 86, 995-1001 came to the same conclusion. However, he apparently was not aware of our recent publications (references 1 and 3) where we stressed the importance of the second Cotton effect in the 325-300 nm region. Even in our first publication (reference 2 to which Kovacs refers, mentioning a need for revision of our proposed rule) we clearly advised measuring the first and the second Cotton effect. Furthermore, we have been unable to reproduce Kovac's results in cases of isoleucine, valine, threonine and glutamic acid, whereas we can consistently reproduce our results.

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