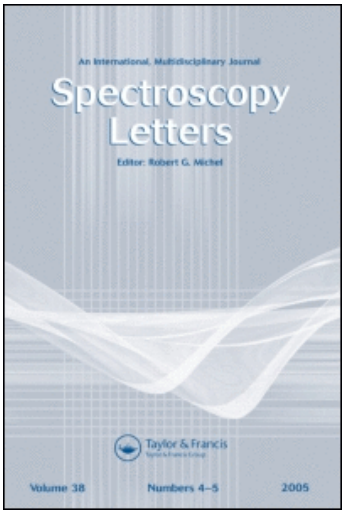

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Chiroptical Properties of Fluorescamine Derivatives of Chiral Primary Amines in situ

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CHIROPTICAL PROPERTIES OF FLUORESCAMINE DERIVATIVES OF CHIRAL PRIMARY AMINES IN SITU

Key Words: CD spectra, absolute configuration, chiral primary amines

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ABSTRACT

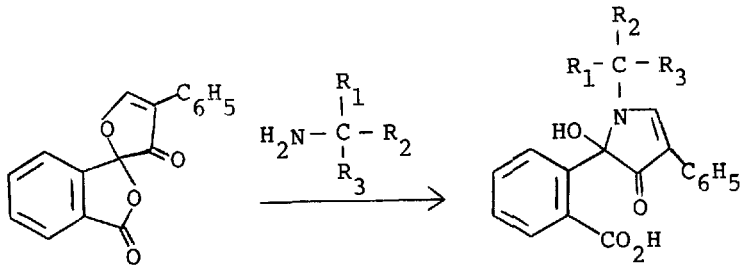
Primary amines react efficiently with fluorescamine (FLURAM®) to form pyrrolinone-type chromophores with long wavelength absorption maxima at 380-410 nm. When the primary amine is chiral, these chromophoric derivatives exhibit characteristic Cotton effects. The chiroptical properties of the reaction mixtures can be related to the absolute configuration of the amines without isolation of the products.

INTRODUCTION

The Cotton effects of the amino chromophores (corresponding to the UV absorption bands[1]) of optically

active aliphatic or alicyclic amines appear in the 195-220 nm region and are not easily accessible with usual ORD/CD instruments. The CD spectra of the arylalkylamines sometimes provide useful stereochemical information[2], but they show multiple Cotton effects, and a change of the substituent on the aromatic ring or a change in its position can reverse the sign and/or alter the intensity of the Cotton effects[3]. Although a quandrant sector rule has been proposed for the 248-270 nm Cotton effects of α- and β-phenylalkylamines[4], it is often advantageous (especially in the case of aliphatic and alicyclic amines) to form chromophoric derivatives whose chiroptical properties can be related to the absolute configuration of chiral amines. The most commonly used chromophoric reagents so far have been salicylaldehyde[5], dimedone[6], 2,4-dinitrochlorobenzene[7], 2-fluoro-3-nitropyridine[8], 2-fluoropyridine-N-oxide[9] and 5-chloro-2-hydroxybenzophenone[10]. Some of the chromophoric derivatives are either difficult to prepare or lead to racemic mixtures[11].

We have recently reported on the application of fluorescamine, 4-phenylspiro(furan-2(3H), 1'-phthalan)-3,3'-dione I as a new chromophoric reagent for the determination of the absolute configuration of primary α-amino acids *in situ*[12]. Chiral primary amines also react efficiently with fluorescamine to form chiroptically active pyrrolinone-type chromophores II:



I II

This reaction is simple and fast and can be performed in test tubes. CD spectra are obtained from the resulting reaction mixtures without isolation of the product.

EXPERIMENTAL

Reagents: Fluorescamine (FLURAM®) and methylbenzylamines (Roche Resolving Agents) were obtained from Hoffmann-La Roche Inc., Nutley, New Jersey, and histological grade dioxane from Fischer Scientific Co., Fair Lawn, New Jersey. All other amines were purchased from Norse Laboratories, Santa Barbara, Calif. The absolute configurations of these chiral amines were determined by Dr. J. W. Westley, Hoffmann-La Roche Inc. via gas-liquid chromatographic (GLC) analysis of the diastereoisomeric N-trifluoracetyl-S-prolyl derivatives[13]. AR-grade chemicals from Mallinckrodt Chemical Works, St. Louis, Mo. were used to prepare the phosphate buffer (pH 8.0, 0.05M).

Method: Two milliliters of a 0.004 M solution of fluorescamine in dioxane is rapidly added to two milli-

liters of a 0.002 M (concentration may range between 10^{-2} and 0.5×10^{-6}M) solution of an amine in 0.05M phosphate buffer pH 8.0. Amines insoluble in aqueous buffer are first dissolved in methanol and then diluted with the phosphate buffer. The reaction mixture is stirred for 15 sec on a Vortex mixer, transferred into a 0.1 cm cell (or into a cell of different length depending on the amine concentration) and the CD spectra are recorded after one min. on a DURRUM-JASCO Spectropolarimeter, Model ORD/CD/UV-5 between 450 and 240 nm. The spectra are difficult to record below 270 nm because of the high absorption of the excess reagent, especially if its concentration is higher than 0.004 M.

RESULTS AND DISCUSSION

As previously reported[14], the optimum pH for the formation of derivatives of primary amines was found to be 8.0. When the amine concentration is in the range of 0.5×10^{-2} to 0.5×10^{-3}M, a twofold excess of fluorescamine is sufficient for the maximum yield *in situ*, but lower amine concentrations require a 20-40 fold excess[12] of the reagent. The reaction is complete within one minute and the chromophore is stable for at least 2 hrs.

The pyrrolinone-type chromophores II derived from the reaction of fluorescamine with primary amines are similar and their absorption maxima are located at

270-280 (ε =18000-20000) and 380-410 nm (ε =6000-7000)[15]. Derivatives of amines containing naphthyl or nitrophenyl groups show additional bands stemming from these aromatic substituents.

A number of chiral primary amines were reacted with fluorescamine and the CD spectra were recorded between 450 and 240 nm. The position and the intensity of the Cotton effects are summarized in Table 1. In Fig. 1 the spectra of R-(+)- and S-(-)-α-methylbenzylamines and S-(+)-2-aminobutan-1-ol reaction products with fluorescamine in situ are shown together with the CD spectra of the synthesized (isolated) chromophoric derivative of R-(+)-α-methylbenzylamine[16]. Both the isolated and in situ-formed chromophoric derivatives have the same spectral shape. The intensity of the Cotton effects of the reaction mixture is about 90% of the isolated standard.

The CD spectra of the in situ reaction mixtures show 3-4 characteristic Cotton effects between 450 and 230 nm, but only those between 450 and 260 nm are readily accessible. The CD curves of the pyrrolinone-type chromophores derived from variously substituted arylalkylamines show a similar pattern. When the amine has an R configuration, the first Cotton effect at 385-411 nm is positive and the Cotton effect at 270-297 nm is negative; an inflection or a weak extremum is ob-

TABLE 1

Cotton Effects in CD Spectra of Reaction Products of Primary Amines with Fluorescamine in Situ[a]

	Amine	1st Cotton Effect		2nd Cotton Effect		3rd Cotton Effect	
		λnm	$(\theta) \times 10^{-3}$	λnm	$(\theta) \times 10^{-3}$	λnm	$(\theta) \times 10^{-3}$
1.	R-(+)-α-Methylbenzylamine	387	+16.52	320(i)	+2.23	275	-24.05
2.	S-(-)-α-Methylbenzylamine	386	-17.00	320(i)	-3.02	276	+24.41
3.	R-(+)-α-Methyl-p-bromobenzylamine	387	+21.62	320(i)	+3.11	276	-38.05
4.	S-(-)-α-Methyl-p-bromobenzylamine	386	-21.45	320(i)	-3.19	275	+40.00
5.	R-(+)-α-Methyl-p-aminobenzylamine	411	+23.85	323(i)	-3.45	297	-25.65
6.	S-(-)-α-Methyl-p-aminobenzylamine	411	-24.21	325(i)	+4.10	296	+26.00
7.	R-(+)-α-Methyl-p-nitrobenzylamine	391	+22.65	340(i)	+4.25	287	-29.75
8.	S-(-)-α-Methyl-p-nitrobenzylamine	390	-23.02	340(i)	-4.16	288	+31.52
9.	R-(+)-α-(1-Naphthyl)-ethylamine[b]	388	+15.62	340(i)	+2.42	287	-26.45
10.	S-(-)-α-(1-Naphthyl)-ethylamine[b]	387	-14.96	341(i)	-1.95	288	+25.96
11.	R-(+)-α-Phenyl-1-propylamine	386	+17.61	321	+5.85	270	-29.55
12.	R-(-)-Norepinephrine .HCl	385	+ 5.12	325	-2.45	285	-11.47
13.	L-(-)-Histidinol .2HCl	388	+16.42	322	-3.20	270	-23.14
14.	R-(-)-2-Aminobutane	385	+ 1.25	320	-2.45	288	- 3.15[c]
15.	R-(-)-2-Aminoheptane	389	+ 7.71	321	-3.16	270	-12.00
16.	S-(+)-2-Aminopropan-1-ol	388	+ 1.15	340	-2.75	285	+ 3.88[c]
17.	S-(+)-2-Aminobutan-1-ol	389	+ 7.05	324	-2.63	284	+ 6.66
18.	R-(-)-2-Aminobutan-1-ol	388	- 6.73	323	+2.25	285	- 5.61

a = In phosphate buffer pH 8/dioxane 1:1 v/v, if not stated otherwise. b = Dissolved in phosphate buffer pH 8/methanol 55:45 v/v. c = The third Cotton effects are difficult to measure because the intensities are low and fluorescamine has a strong absorption in this area. i = Inflection.

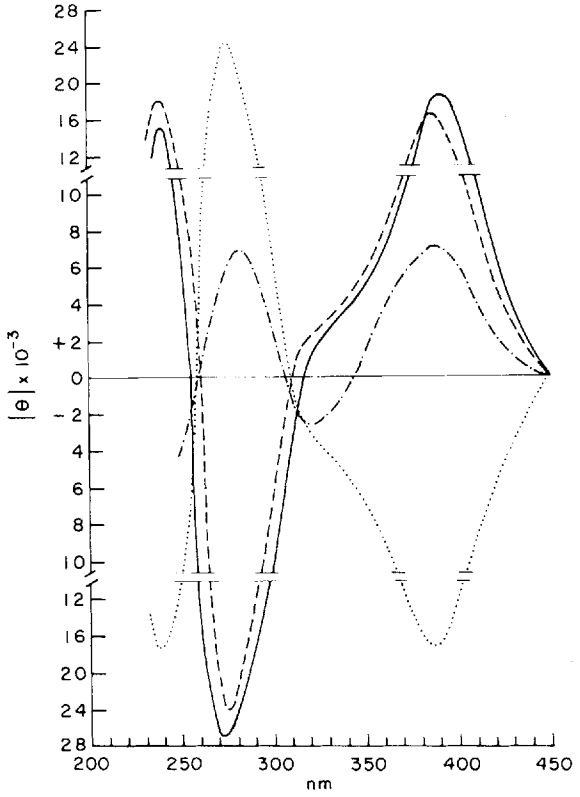


FIG. 1

CD spectra of a synthesized (isolated) chromophoric derivative of R-(+)-α-methylbenzylamine (——) with fluorescamine in ethanol and of the in situ reaction mixtures of R-(+)-(----) and S-(-)-(.....)- -methylbenzylamine and S-(+)-2-amino-1-butanol (-·-·-) with fluorescamine in phosphate buffer pH 8/dioxane 1:1 v/v.

served in the 320-340 nm region. Within the experimental error, the CD spectra of the chromophores derived from the S-arylalkylamines are mirror images of those derived from R-arylalkylamines (Table I, Fig. 1).

Entries 12 and 13 are of particular interest because the amino group is not at the asymmetric center, but it is separated from it by a methylene group, suggesting the possibility of using fluorescamine condensation compounds with amines to determine the stereochemistry of more remote asymmetric centers.

The CD spectra of the fluorescamine derivatives with chiral alkylamines investigated so far show Cotton effects in the same general wavelength region as described above for arylalkylamines, but their intensities are much lower. This may be caused by the increased conformational mobility or by the absence of electronic interaction between the aromatic moiety of the amine and the pyrrolinone-type chromophore. The first Cotton effect of the R-2-aminobutan-1-ol is negative, but by replacing the CH_3 group with a CH_2OH group the S sequence of the amine changes to R-assignment[17] without a change of the chemically correlated absolute configuration. Therefore, when using the R and S sequence rule for the determination of the absolute configuration of chiral amines based on the chiroptical properties of their fluorescamine condensation

compounds, one is probably restricted to the molecules which are close structural analogs. Otherwise one must be aware that a change of R or S designation based on the fiducial precedence of a new substitute may not always correspond to the chemically derived absolute configuration of a chiral amine.

The main advantage of this fluorescamine method is its simplicity and its mild reaction conditions. Routinely as little as 0.1-1.0 μg/ml of a chiral amine has been reacted with fluorescamine and useful CD spectra were directly from such mixtures. For the determination of absolute configuration of more complex chiral amines it may be necessary to isolate and purify the chromophoric derivative[15].

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