CHIROPTICAL PROPERTIES OF FLUORESCAMINE CONDENSATION COMPOUNDS WITH CHIRAL SECONDARY AMINES IN SITU†

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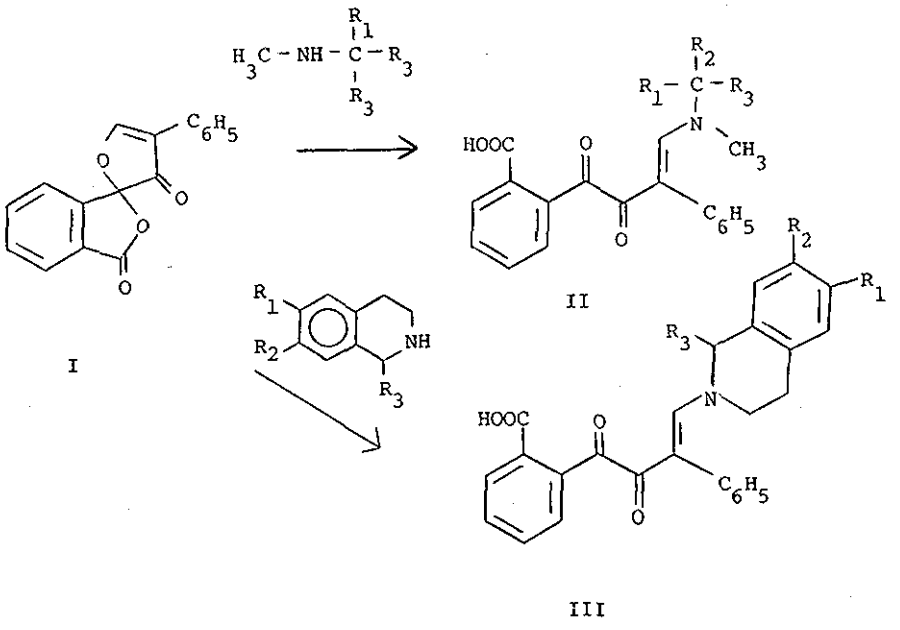
Chiral secondary amines react readily with fluorescamine (FLURAM®) to form aminoenone-type chromophores with long wavelength absorption maxima at 300-320 nm. The chiroptical properties of the reaction mixtures reflect the absolute configuration of secondary amines in situ.

We have recently described the application of fluorescamine, 4-phenylspiro (furan-2(3H),1'-phthalan)-3,3'-dione (I) as a chromophoric reagent for the determination of the absolute configuration of chiral primary amines in situ[1]. Fluorescamine also reacts efficiently with chiral acyclic and cyclic secondary amines[2,3,4] to form aminoenone-type chromophores II or III. These chromophores are chiroptically active and CD spectra can be obtained from the reaction mixtures without isolation of the products. This reaction is simple and fast and can be performed in test tubes under mild conditions. Table I shows a number of secondary amines which were reacted with fluorescamine.

† Dedicated to Professor Robert Burns Woodward on the occasion of his sixtieth birthday.



I

II

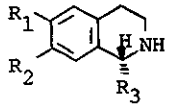
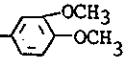
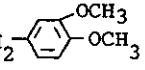
III

In a general procedure 2 ml of a *0.004 M solution of fluorescamine* (FLURAM ®)in dioxane are rapidly added to 2 ml of a 0.002 M (concentration may range between 10^{-2} and 0.5 x 10^{-6} M) solution of an amine in 0.05 M phosphate buffer pH 8 (amines insoluble in buffer were first dissolved in methanol). After stirring for 15 sec on a Vortex mixer and standing for 7 min, the CD spectra were recorded on a JASCO Spectropolarimeter, *Model J-20* between 450 and 240 nm[5].

The aminoenone-type chromophores II or III show UV maxima at 250-255 nm (ε=ca 14,500) and 300-320 nm (ε=16,000-20,000); an inflection is observed at 340-360 nm (ε=6,000-10,000).[2,3,6]

The CD spectra of the reaction mixtures show 3-4 *bands between* 360 and 240 nm, but only those between 360 and 290 nm are readily access-

Table 1. 1st and 2nd Cotton Effects in CD Spectra of Reaction Products of Secondary Amines with Fluorescamine in Situ (i=inflection)

	Secondary Amine	1st λ nm	1st [θ] x 10^{-3}	2nd CD Bands[a] λ nm	2nd CD Bands[a] [θ] x 10^{-3}
	Acyclic Amines[7]				
1	L-(-)-Ephedrine (1R,2S)	343	+6.81	312	-19.21
2	D-(+)-Ephedrine (1S,2R)	343	-6.35	312	+19.00
3	R-(-)-Desoxyephedrine .HCl	345	-6.51	311	+31.05
4	R-(+)-N,α-Dimethylbenzylamine	340(i)	+6.60	308	+17.14
5	S-(-)-N,α-Dimethylbenzylamine	340(i)	-7.19	307	-18.60
	Cyclic amines[8]				
6					
6a	(R):R_1=R_2=OCH_3,R_3=CH_3	340	-2.62	295	-15.56
6b	(S)-configuration of 6a	341	+3.39	295	+16.25
6c	(R):R_1=R_2=$H_1$$R_3$=$CH_2$ 	351	+1.41	311	-40.00
6d	(S)-configuration of 6c	350	-1.21	310	+39.50
6e	(R):R_1=R_2=OCH_3,R_3=CH_2 	346	+2.00	312	-32.25
6f	(S)-configuration of 6e	352	-1.79	312	+32.56

a) [θ] are reported in reference to the molar concentrations of secondary amines.

ible and do not overlap with CD bands stemming from other chromophoric groups which may be present; e.g. dimethoxyphenyl. Therefore only the positions and the intensities of the first and second Cotton effects are summarized in Table 1. In Figure 1 the CD spectra of the L- and D-ephedrine derivatives are shown along with that of 6f.

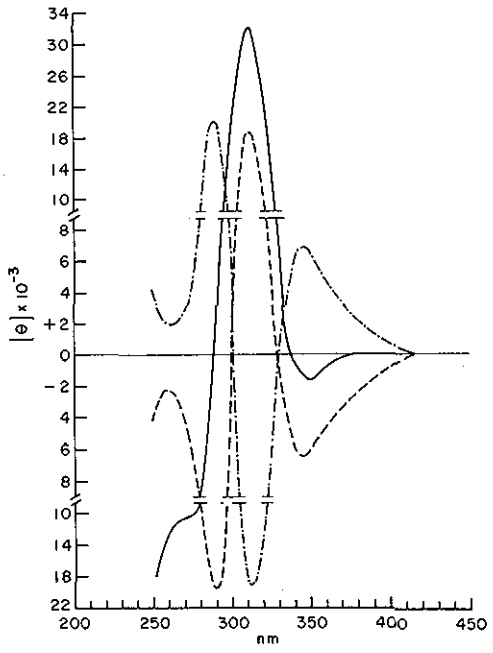


Fig. 1 CD spectra of the <u>in situ</u> reaction mixtures of L-(1R,2S)-(-·-) and D-(1S,2R)-(---)-ephedrine and compound 6f (——) with fluorescamine in phosphate buffer pH 8/dioxane 1:1 v/v.

The first CD bands in the 340-360 nm area (corresponding to the inflection in the UV spectrum) are relatively weak and appear sometimes as an inflection. The second Cotton effects observed in the 295-316 nm region are

the strongest (corresponding to the main UV maxima) and they are negative for chromophores derived from acyclic secondary amines of S configuration (compounds 1 and 5) and positive in the case of cyclic secondary amines of S-configuration (compounds 6b, 6d and 6f). Within the experimental error, the CD curves of the chromophores derived from S-amines are mirror images of those of the corresponding R-amine derivatives.

The opposite sign of the main Cotton effects of the chromophoric derivatives of acyclic and cyclic secondary amines of the same absolute configuration may be caused by different conformation of the amine moiety in solution (due to steric requirements)[9]. More experimental data are needed for generalization and interpretation of this empirical rule. Therefore, configuration assignments based on this study should be restricted to molecules which are close structural analogs.

As in the case of primary amines, the main advantage of this fluorescamine method is its simplicity.

REFERENCES AND NOTES

1 V. Toome, B. Wegrzynski, and J. Dell, Spectroscopy Letters, 1977, 10, 000, in press and references therein.

2 M. Weigele, S. L. DeBernardo, J. P. Tengi, and W. Leimgruber, J. Amer. Chem., 1972, 94, 5927.

3 V. Toome and K. Manhart, Analytical Letters, 1975, 8, 441.

4 A. F. Felix, V. Toome, S. DeBernardo, and M. Weigele, Arch. Biochem. Biophys., 1975, 168, 601.

5 For experimental details see ref. 1.

6 In the presence of dimethoxyphenyl-type chromophores, additional maxima or shoulders are observed in the 270-280 nm area.

7 D- and L-ephedrine were purchased from Aldrich Chemical Co. Inc., Milwaukee, Wisconsin and desoxyephedrine hydrochloride from Sumner Chemical Co., Div. of Miles Laboratories, Elkhart, Indiana; N,α-dimethylbenzylamines (Roche resolving agents) were obtained from Hoffmann-La Roche Inc., Nutley, New Jersey.

8 Compounds 6a-6f were synthesized by S. Teitel et al., Hoffmann-La Roche Inc., Nutley, New Jersey. Their absolute configurations have been described by S. Teitel, J. O'Brien, and A. Brossi, J. Med. Chem., 1972, 15, 845; S. Teitel and J. O'Brien, Heterocycles, 1974, 2, 625.

9 Temperature and solvent studies are planned to clarify this assumption.

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