CHIRAL PRODYES : SYNTHESIS AND FULL CHARACTERIZATION OF (S)-1-PHENYLETHYLAMIDES OF THE OPTICALLY ACTIVE O-METHYLDIHYDROFLUORESCEINS

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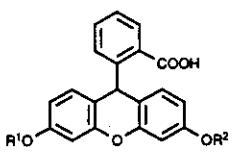
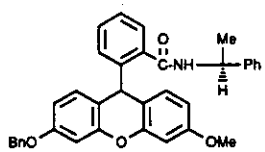
Abstract - O-Dihydrofluorescein (3) on reaction with (S)-(-)-1-phenylethylamine afforded amide (5) as a mixture of diastereomers which resisted chromatographic separation. The mixture of amides (6) and (7), however, obtained from 5 by catalytic hydrogenation could be separated by hplc. The X-ray analysis of amide (6) showed that it has the (9S)-absolute configuration. This result attests that it is possible to obtain dihydrofluoresceins with differently substituted benzene rings in the form of enantiomers.

Dihydrofluorescein diacetate (1,DADF) and O,O-dibenzyldihydrofluorescein (2,DBDF) are reagents useful to characterize and to quantitate amines and alcohols.[1-3] The DADF-derivatives convert on tlc plates on exposure to ammonia and iodine vapors into red colored erythrosine derivatives, which exhibit uv maxima at 540 nm (EtOH). A similar reaction takes place when the DBDF-derivatives are deblocked by catalytic hydrogenation, and the phenolic products exposed to iodine vapors on tlc plates. It was our plan to develop chiral representatives of this series of compounds which could be of a wide application in histochemistry. This is our first report on the results achieved in accomplishing this goal.



1 $R^1 = R^2 = Ac$
2 $R^1 = R^2 = Bn$
3 $R^1 = Bn$, $R^2 = Me$
4 $R^1 = H$, $R^2 = Me$



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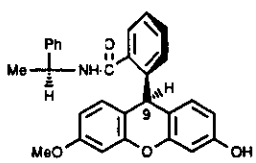

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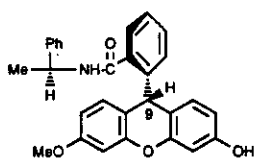

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Dihydrofluoresceins with different substituents in the benzene rings of the xanthene moiety make C(9) a chiral carbon atom and these compounds, therefore, should exist as enantiomers. This, with the accomplished synthesis of amides (6) and (7), proved to be the case. Dihydrofluorescein (3)[3], on reaction with (S)-(-)-1-phenylethylamine of known absolute configuration afforded amide (5) as a mixture of diastereomers. It was not possible to separate 5 on tlc, or by hplc, on using a variety of solvent systems. However, a clean separation was accomplished, after catalytic hydrogenation of amide (5) over $Pd(OH)_2$ catalyst in ethyl acetate solution, by hplc. Amide (6) which separated as the less polar material (retention time 8.3 min) and more polar amide (7) (retention time 10.8 min) was cleanly separated. Amide (6), mp 109-110 °C, $[\alpha]_D$ - 7.3° ($CHCl_3$), was found suitable for an X-ray analysis, and details are summarized below. Amide (7) remained amorphous.

X-RAY ANALYSIS OF (9S)-O-METHYLDIHYDROFLUORESCEIN-(S)-1-PHENYLETHYLAMIDE (6): The X-ray results showed that the fused-ring moiety is made up of two planar segments having the ether oxygen and C-9 in common and making an angle of 7.5° with one another. The terminal phenyl ring is approximately coplanar with the fused ring system while the central phenyl ring is approximately perpendicular to it. Since it was known that the compound contained (S)-(-)-phenylethyl amide, the X-ray study indicates that the configuration at C-9 is also (S). There is one intermolecular hydrogen bond in which the hydroxyl oxygen is a donor to the carbonyl oxygen. The co-crystallized isopropanol solvent molecule also participates in hydrogen bonding being a donor to the hydroxyl oxygen in 6 and an acceptor from the NH moiety of a second molecule of 6 .

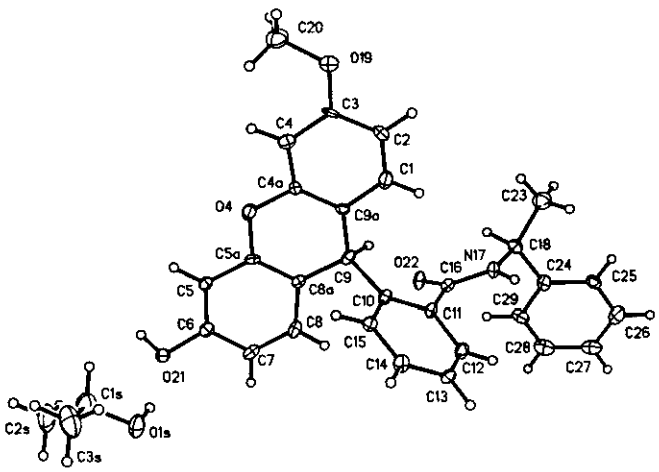

Figure 1: Diagram showing the structure and conformation of 6 . The included solvent molecule is also shown. The figure is drawn using the experimentally determined coordinates with the thermal ellipsoids at 20% probability.

X-ray crystallographic data for 6: $C_{29}H_{25}NO_4 \cdot C_3H_8OH$, molecular weight = 511.6, orthorhombic, space group $P2_12_12_1$, a = 8.742(2), b = 11.276(2), c = 27.872(4) Å, d_{calc} = 1.24 g cm^{-3}, μ = 0.63 mm^{-1}, 1845 independent reflections were measured out to $2\theta_{max}$ = 105° with a Nicholet R3M diffractometer using CuKα radiation (λ = 1.54178 Å) with a graphite monochromator in the incident beam. The data were collected at -50 °C using the $\theta/2\theta$ scan technique with a variable ω scan rate ranging from

8°/min minimum to 30°/min maximum, depending upon the intensity of a reflection. The structure was solved by direct methods as implemented by the SHELXTL system of programs.[4] Full-matrix least-squares refinement on 356 parameters (coordinates and anisotropic thermal parameters for nonhydrogen atoms; coordinates only for hydrogen atoms on C-9, N-14, C-18 and O-21; remaining hydrogen atoms placed at calculated positions, C-H = 0.96 Å, and allowed to ride on covalently bonded atoms), using the 1642 reflections for which $|F_o|>3\sigma|F_o|$ gave a final R-factor of 5.2% (R_w = 6.0%). The goodness of fit parameter was 1.77 and the final difference map was featureless. Tables of coordinates, bond length and angles have been deposited with the Crystallographic Data Center, Cambridge University Chemical Laboratory, Cambridge CB2 1EW, England.

It was not possible to hydrolyze amides (6) and (7) with methanolic sodium hydroxide, or with sodium hydroxide in ethylene glycol, or with methanolic hydrochloric acid under reflux. The preparation of the enantiomers of O-methyldihydrofluorescein (4) has to await the results of further experimentation. However, with the full characterization of amides (6) and (7) and the X-ray analysis of the (9S)-configurated amide (6), it is demonstrated that dihydrofluoresceins which are differently substituted in the benzene rings of the xanthene moiety, can be obtained as enantiomers. It can be envisaged that optically active prodyes also exist in other classes of compounds, such as triarylcarbinols, the leukobases of triarylmethane dyes.[5]

EXPERIMENTAL

Melting points (uncorrected): Fisher-Johns apparatus; optical rotations ($[\alpha]_D$, $CHCl_3$): Perkin-Elmer-241 MC automatic polarimeter; ir spectra (cm^{-1},$CHCl_3$): Beckman-IR-4230 instrument; uv spectra were measured in EtOH (λ_{max}, nm): Hewlett-Packard 8450 UV spectrophotometer; ^1H nmr (in $CDCl_3$ with Me_4Si as internal reference, δ ppm, J Hz): Varian XL-300, Gemini 300 MHz spectrometers; ms (m/z) for chemical ionization (CI): Finnigan-1015D mass spectrometer; ms (m/z) for electron-impact (EI): V.G. Micromass 7070F mass spectrometer; thin layer chromatography (silica gel GHLF, 250 μm): Analtech Inc.; column chromatography (silica gel GHLF, 250 μm): Merck 60 (230-400 mesh); the solvent systems used for tlc analysis were the following: CH_2Cl_2; CH_2Cl_2/5% MeOH; the solvent system used for column chromatography: CH_2Cl_2/10% MeOH; preparative hplc was performed on an Axxiom silica column (5 μ, 10 x 250 mm), using 0.6% MeOH in CH_2Cl_2 at a flow rate of 5 ml/min. O-Benzyl-O-methyldihydrofluorescein (3) was prepared in three steps from O-benzylfluorescein benzyl ester.[3]

(±)-Benzyl-O-methyldihydrofluorescenylphenylethylamide (5): To a solution of O-benzyl-O-methyldihydrofluorescein (1.58 g, 3.56 mmol) in dry CH_2Cl_2 (100 ml), (S)-(-)-1-phenylethylamine (0.42 g, 3.46 mmol), DCC (0.73 g, 3.56 mmol) and 4-(N,N-dimethylamino)pyridine (0.5 g) were added. The reaction mixture was stirred at room temperature for 18 h and then filtered. The filtrate was washed with 1N HCl (2 x 20 ml), twice with 10% aq. $NaHCO_3$ and finally with brine. Evaporation of solvent after drying over anhydrous Na_2SO_4 gave 1.46 g of a yellow foam. Chromatography of the product on a silica gel column (CH_2Cl_2, CH_2Cl_2:MeOH-10%) gave 1.30 g (69%) of tlc pure product as a yellowish amorphous solid. : CI ms (m/z): 542 (M^++1); EI ms (m/z): 541 (M^+); ir: 3420, 1640, 1500; uv: 230, 278; ^1H nmr (mixture of diastereomers; some signals are doubled, that is the apparent multiplicities are either real or are a result of superimposition of signals from the two diastereomers): 1.63 (d, J = 6.8, 3H), 3.78, 3.79 (s each, 3H each), 5.03, 5.04 (s each, 2H each), 5.38 (quint, J_{app} = 7.5, 1H), 5.72 (s, 1H), 6.16 (d, J = 8.0, NH), 6.45 (dd, J_o = 8.5, J_m = 2.6, 1H), 6.52 (dd, J_o = 6.3, J_m = 2.6, 1H),

6.54 (dd, J_o = 6.1, J_m = 2.5, 1H), 6.60 (d, J = 2.8, 1H), 6.62 (br d, J = 2.4, 1H), 6.71 (t, J_{app} = 2.4, 1H), 6.91 (d, J = 8.3, 1H), 7.07 (br d, J = 9.4, 1H), 7.14 (dt, J_o = 7.4, $J_{app,m}$ = 1.1, 1H), 7.22 (dd, J_o = 7.6, $J_{app,m}$ = 1.1, 1H), 7.26-7.44 (remaining aromatic 12H). Anal. Calcd for $C_{36}H_{31}NO_4$: C, 79.83; H, 5.77; N, 2.58. Found: C, 79.85; H, 5.75; N, 2.55.

(±)-O-Methyldihydrofluorescenylphenylethylamides (6) and (7): To a solution of 5 (1.30 g, 2.40 mmol) in 300 ml of EtOAc, 0.65 g of $Pd(OH)_2$ on carbon was added and the reaction mixture was hydrogenated at room temperature with stirring for 1.5 h. The mixture was filtered and the solvent was evaporated to give 1.10 g of a yellow oil. Filtration of the reaction mixture through a silica gel column (CH_2Cl_2:MeOH-10%) gave 1.0 g (92%) of a yellow foam. Tlc of this product (CH_2Cl_2:MeOH-5%) showed two spots (corresponding to two diastereomers, $[\alpha]_D$ -7.5°, c = 0.5, $CHCl_3$). Preparative hplc of the mixture of diastereomers (0.40 g) on an Axxion silica column using 0.6% MeOH in CH_2Cl_2 at a flow rate of 5 ml/min gave 0.19 g of compound (6) and 0.17 g of compound (7), each as a yellow foam. Yellow crystals of 6 were obtained upon crystallization from *i*-PrOH.

(9S)-(-)-Methyldihydrofluorescenylphenylethylamide (6): mp 109-110 °C, $[\alpha]_D$ -8.8° (c = 0.5, $CHCl_3$), Cl ms (m/z): 452 (M^+ + 1); El ms (m/z): 449 (M^+ - 2); ir: 3600, 3320, 1650, 1500; uv: 215, 279; ^1H nmr: 1.62 (d, J = 7.0, 3H), 3.78 (s, 3H), 5.25 (br s, OH), 5.36 (quint, J = 7.4, 1H), 5.69 (s, 1H), 6.18 (d, J = 8.0, NH), 6.36 (dd, J_o = 8.5, J_m = 2.6, 1H), 6.54 (dd, J_o = 8.5, J_m = 2.6, 1H), 6.57 (d, J_o = 2.6, 1H), 6.62 (d, J_m = 2.6, 1H), 6.87 (d, J_o = 8.5, 1H), 7.01 (d, J_o = 8.5, 1H), 7.06 (dd, J_o = 7.8, J_m = 1.1, 1H), 7.15 (dt, J_o = 7.5, J_m = 1.3, 1H), 7.23 (dd, J_o = 7.5, J_m = 1.3, 1H), 7.28-7.44 (remaining aromatic 6H). Anal. Calcd for $C_{29}H_{25}NO_4.C_3H_8OH$: C, 75.12; H, 6.70; N, 2.73. Found: C, 74.62; H, 6.47; N, 2.73.

(9R)-(+)-Methyldihydrofluoresceylphenylethylamide (7): Amorphous solid, $[\alpha]_D$ +10.8° (c = 0.5, $CHCl_3$); Cl ms (m/z): 452 (M^+ + 1), El ms (m/z): 449 (M^+ - 2); ir: 3610, 3320, 1650, 1500; uv: 220, 278; ^1H nmr: 1.62 (d, J = 7.0, 3H), 3.78 (s, 3H), 5.36 (quint, J = 7.4, 1H), 5.69 (s, 1H), 6.16 (d, J = 8.0, NH), 6.44 (br dd, J_o = 8.5, J_m = 2.6, 2H), 6.58 (d, J_m = 2.6, 1H), 6.63 (d, J_m = 2.6, 1H), 6.87 (d, J_o = 8.5, 1H), 7.00 (d, J_o = 8.5, 1H), 7.06 (dd, J_o = 7.8, J_m = 1.1, 1H), 7.15 (dt, J_o = 7.5, J_m = 1.3, 1H), 7.23 (dd, J_o = 7.5, J_m = 1.3, 1H), 7.27-7.42 (remaining aromatic 6H). Anal. Calcd for $C_{29}H_{25}NO_4$: C, 77.14; H, 5.58; N, 3.10. Found: C, 76.76; H, 5.84; N, 2.84.

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