35. Reaction of Alcohols and Amines with Diacetyldihydrofluorescein (DADF): Conversion into Erythrosine-Derivatives on TLC-Plates by Ammonia and Iodine Vapors

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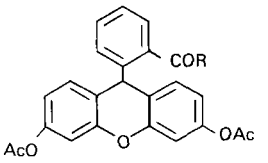
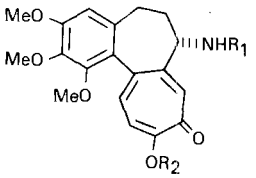
Summary

Reaction of deacetylcolchicine (**2**) and colchifoline (**3**) with diacetyldihydrofluorescein (**1**, DADF) afforded the corresponding amide and ester derivatives, converted on TLC-plates after exposure to ammonia and iodine vapors into red colored pigments. This reaction, also observed with DADF-derivatives of codeine, quinine and mescaline is highly sensitive. The red pigment produced from the DADF-ester (**6**) of colchifoline formed by the ammonia-iodine treatment is the corresponding erythrosine ester derivative. DADF emerges from these investigations as a useful reagent to detect alcohols and amines in crude mixtures and for dye labeling.

Diacetyldihydrofluorescein (**1**, DADF) is a well known compound [1], and easily available from fluorescein by reduction with Zn/NH_4Cl in refluxing EtOH, followed by acetylation of the dihydrocompound with Ac_2O in pyridine. A TLC analysis of DADF afforded red colored spots after exposure of the material on plates to ammonia and iodine vapors suggesting that a similar color reaction might also occur with derivatives of DADF. In search for high affinity probes to map the colchicine binding site on tubulin [2] [3], we considered it worthwhile to prepare DADF-derivatives of the biologically potent colchinoids, deacetylcolchicine (**2**) and colchifoline (**3**) [3], hoping that they would bind to tubulin protein and could then be made visible in free and bound form by the color reaction. Reaction of **2** and **3** with DADF in dry CH_2Cl_2 in the presence of dicyclohexylcarbodiimide and a catalytic amount of 4-dimethylaminopyridine [4] afforded the crystalline amide **5** and the ester **6**, respectively. Treatment of the amide **5** with aqueous ammonia afforded the diphenol **7**, further converted with H_2O_2 in ethanol into a yellow-green fluorescent dye with properties similar to fluorescein. Although **5** and **6** did not bind to tubulin protein sufficiently (15 and 30%, respectively) [5], after chromatography and exposure of the plates to ammonia and iodine vapors they both developed red colors similar to those already observed with DADF. The crude dyes obtained from **5** and **6** after extraction from the silica gel TLC plates contained large amounts of iodine and showed UV maxima at 546 nm in EtOH, suggesting that the reactions on plates possibly involved conversion of the DADF-part

of the molecule into erythrosine. This is now supported by the following data. Rechromatography of the red dye obtained from the ester **6** and separation from a slower moving yellow spot afforded an optically active red pigment which was contaminated with inorganic material (silica gel). Its UV maximum at 546 nm and its IR, NMR spectra and mass spectra were in good agreement with structure **8**, representing the erythrosine-ester of colchifoline. Furthermore, hydrolysis of **8** with 2N NaOH afforded two compounds which were identical by TLC comparison with erythrosine and colchifoleine (**4**). It seems now reasonably ascertained that the reactions occurring on TLC plates by the exposure of **6** to ammonia and iodine vapors involved first hydrolysis of the two aromatic AcO-groups, followed by iodination and oxidation of the diphenolic intermediate by iodine.





1 DADF, R = OH

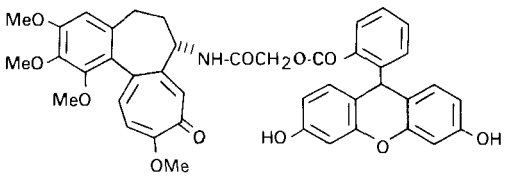
5 DADF-amide of **2**

6 DADF ester of **3**

2 $R^1 = H$, $R^2 = Me$

3 $R^1 = COCH_2OH$, $R^2 = Me$

4 $R^1 = COCH_2OH$, $R^2 = H$



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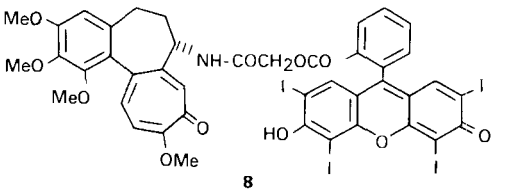

8

↓ 2N NaOH

4 + Erythrosine

DADF afforded the corresponding derivatives with codeine, quinine and mescaline which were fully characterized by spectral data. They were converted on TLC plates after exposure to ammonia and iodine vapors into red pigments similar to those already obtained from **5** and **6**. A dilute solution of DADF in CH_2Cl_2 showed the reac-

tion to be sensitive up to a 0.1% concentration of the substrate. DADF may be useful for the detection of alcohols and amines in the crude mixtures, detectable on TLC plates after treatment with ammonia and iodine vapors as their erythrosine derivatives, and a good pro dye labelling agent.

We would like to thank Dr. *Henry M. Fales* for the mass spectrum of *O*-Erythrosylcolchifoline, and Prof. *Lester A. Mitscher* for reading the manuscript prior to publication.

Experimental Part

General. The melting points (m.p.) were taken on a Fisher-Johns apparatus and are uncorrected. TLC plates (silica gel) were purchased from *Analtech,* Inc., New York D.E. Optical rotations were measured by using a *Perkin-Elmer* Model *241 MC* polarimeter with solvents and concentrations specified. UV spectra were measured on *Hewlett Packard 8450A* spectrophotometer in EtOH. IR spectra were obtained on a *Beckman 4230* instrument (cm^{-1}) in $CHCl_3$, if not otherwise stated. ^{1}H-NMR spectra were measured in $CDCl_3$, if not otherwise stated by a *Varian HR-220* spectrometer relative to TMS as internal reference. CI-MS spectra (*m/z*) were obtained with a Finnigan 1015D spectrometer, and EI-MS spectra were recorded with a *Hitachi Perkin-Elmer RMU-6E* spectrometer (70 eV). Elemental analysis were performed by the *Seciton* on Microanalytical Services and Instrumentation of this laboratory.

Dihydrofluorescein. To a refluxed heterogeneous suspension of NH_4Cl (12 g, 0.22 mol), fluorescein (**1**, 3.5 g, 0.47 mmol) in abs. EtOH (80 ml) was added Zn (1.8 g, 0.027 mol) in batches. The reaction mixture was refluxed overnight, filtered and the filtrate extracted with a mixture of $CHCl_3$ i-PrOH (3:1, 4 × 20 ml). The combined org. layer was dried (Na_2SO_4), concentrated, to afford an amorphous yellowish residue of dihydrofluorescein (3.4 g), CI-MS: 335 ($M^+ + 1$).

Diacetyldihydrofluorescein (DADF, **1***):* A solution of the amorphous yellowish residue of dihydrofluorescein (3.4 g, 10.17 mmol) in pyridine (45 ml) and Ac_2O (15 ml) was stirred at r.t. overnight. The mixture was concentrated *i.v.* to afford a white solid residue. This material was purified by passing through a column of silica gel and eluted with a mixture of $CHCl_3$/MeOH (98:2), to afford a white solid, which on trituration with benzene afforded a pure white solid **1** (4 g, 94%): m.p. 213° ([1]: 213°). IR: 1760, 1720 (2AcO) and 1610 (arom.) ^{1}H-NMR: 2.15 (*s,* 6H, 2AcO); 6.32–8.00 (*m,* 11H, 10 arom. H and CH); EI-MS: 418 (M^+). Anal. calc. for $C_{24}H_{18}O_7 \cdot \frac{1}{2} H_2O$ (427.42): C 67.44, H 4.48; found: C 67.80, H 4.70.

(Diacetyldihydrofluoresceyl)deacetylcolchicine (**5**). A solution of DADF (**1**, 350 mg, 0.83 mmol), deacetylcolchicine (**2**, 350 mg, 0.98 mmol), dicyclohexylcarbodiimide (DCC) (350 mg, 1.69 mmol) and 4-(dimethylamino)pyridine (30 mg) in dry CH_2Cl_2 (8 ml) was stirred at r.t. for 2 h. The mixture was filtered and washed with dry CH_2Cl_2 (2 × 0.5 ml). The org. layer was washed with 0.5N HCl (3 × 1 ml), 10% aq. $NaHCO_3$ (3 × 1 ml) and H_2O (2 × 1 ml), dried (Na_2SO_4) and evaporated to afford a solid residue, which was crystallized with MeOH to afford **5** (340 mg, 65%): m.p. 186°; $[a]_D^{25} = -102°$ ($c = 0.25$, $CHCl_3$). IR: 1750 (AcO) and 1620 (arom.). ^{1}H-NMR: 2.16 (*s,* 3H, AcO); (*s,* 3H, AcO); 1.76–2.72 (*m,* 4H, 2 CH_2); 3.68 (*s,* 3H, MeO); 3.82 (*s,* 3H, MeO); 3.94 (*s,* 3H, MeO); 3.96 (*s,* 3H, MeO); 5.76 (*s,* 1H, CH) and 6.36–7.48 (*m,* 14H, 14 arom. H). CI-MS: 758 ($M^+ + 1$). Anal. calc. for $C_{44}H_{39}NO_{11} \cdot H_2O$ (775.82): C 68.11, H 5.32, N 1.80; found: C 67.94, H 5.54, N 1.72.

(Dihydrofluoresceyl)deacetylcolchicine (**7**). To a stirred solution of **5** (245 mg, 0.32 mmol) in MeOH (8 ml) was added dropwise a conc. aq. solution of NH_3 (8 ml) and the mixture stirred for 2 h, until TLC showed the absence of **5**. The mixture was concentrated under reduced pressure to yield a yellowish solid residue, which was crystallized from a mixture of CH_2Cl_2/Et_2O to afford pure **7** (200 mg, 93%); m.p. 205° (dec.); $[a]_D^{25} = -127°$ ($c = 0.3$, MeOH). IR: 1600 (arom.). ^{1}H-NMR ($CDCl_3 + CD_3OD$): 1.92–2.64 (*m,* 4H, 2CH_2); 3.64 (*s,* 3H, MeO); 3.80 (*s,* 3H, MeO); 3.82 (*s,* 3H, MeO); (*s,* 3H, MeO); (*s,* 3H, MeO); 5.36 (*s,* 1H, CH), 6.36–7.52 (*m,* 14H, 14 arom. H); CI-MS: 674 ($M^+ + 1$). Anal. calc. for $C_{40}H_{35}NO_9$ (625.73): C 76.78, H 5.63, N 2.23; found: C 76.38, 5.77, N 1.83.

Fluoresceyldeacetylcolchicine. To a solution of **7** (2 mg, 0.002 mmol) in EtOH (0.05 ml) was added dropwise a 37% aq. solution of H_2O_2 (0.5 ml) and the mixture was stirred overnight at r.t. The mixture showed a greenish fluorescence similar to fluorescein. CI-MS: 672 ($M^+ + 1$).

(Diacetyldihydrofluoresceyl)colchifoline (**6**). A solution of colchifoline (**3**, 365 mg, 0.87 mmol), DADF (**1**, 365 mg, 0.87 mmol), DCC (365 mg, 1.77 mmol), 4-(dimethylamino)pyridine (20 mg) in dry CH_2Cl_2 (3 ml) was stirred at r.t. for 2 h. The mixture was filtered, washed with 10% $NaHCO_3$ (3 × 3 ml), 0.5N HCl (3 × 3 ml), H_2O (2 × 1 ml), dried (Na_2SO_4), concentrated to afford a residue, which was purified by column chromatography over silica gel. Elution with CH_2Cl_2/MeOH (99:1) afforded a pure solid **6** (400 mg, 77%): m.p. 171°; $[\alpha]_D^{25} = -60°$ (c = 0.3, $CHCl_3$). IR: 1750 (AcO) and 1600 (arom.). ^{1}H-NMR: 1.64–2.52 (*m*, 6H, $2CH_2$); 2.28 (*s*, 6H, 2AcO); 3.64 (*s*, 3H, MeO); 3.90 (*s*, 3H, MeO); 3.94 (*s*, 6H, 2MeO); 4.84 (*s*, 1H, CH); 6.32–7.92 (*m*, 14H, 14 arom. H). CI-MS: 816 ($M^+ + 1$). Anal. calc. for $C_{46}H_{41}NO_{13} \cdot 1\frac{1}{2}\ H_2O$ (842.86): C 65.55, H 5.26, N 1.66; found: C 65.49; H 5.50, N 1.59.

Treatment of **6** *with NH_3 and Iodine Vapors. – O-Erythrosylcolchifoline* (**8**). Compound **6** was subjected to silica gel prep. TLC and developed with CH_2Cl_2/MeOH (95:5), exposed to NH_3 vapors for 15 min followed by exposure to iodine vapors for 15 min after drying. The pink spot was scraped out and extracted with CHCl i-PrOH (3:1, 3 × 10 ml). The combined org. layer was evaporated under reduced pressure to afford a pink solid, which was purified by re-chromatography over silica gel prep. plates with CH_2Cl_2/MeOH (85:15), giving a pink solid **8** (150 mg, 29%): m.p. 285°; $[\alpha]_D^{25} = 146°$ (c = 0.17, MeOH). UV: 350 (2.33), 510 (2.53) and 546 (2.97). IR: 3380 (OH), 1600 (arom.). ^{1}H-NMR (CD_3OD): 2.56–3.72 (*m*, 6H, $3CH_2$); 3.84 (*s*, 6H, 2MeO); 3.92 (*s*, 3H, MeO); 3.96 (*s*, 3H, MeO); 4.56 (br. *s*, 1H, CH); 6.70–8.40 (*m*, 9H, 9 arom. H). 252 cf MS: 1278.55 for ($M^+ + 2Na - H$) (calc. 1278.30); 1255.79 ($M^+ + Na$) (calc. 1256.37); 1152.70 for ($M^+ - I + 2Na$) (calc. 1152.40); 1130.22 for ($M^+ - I - H + Na$) (calc. 1130.43).

Hydrolysis of **8**. A solution of **8** (10 mg) in MeOH (0.5 ml) and 2N NaOH (0.05 ml) was stirred at r.t. for 2 h. The mixture was concentrated under reduced pressure to afford a red residue. TLC of this residue developed with CH_2Cl_2/MeOH (99.1) showed 2 spots (yellow and pink) corresponding to authentic samples of colchifoleine (**4**), and erythrosine, respectively. CI-MS: 402 ($M^+ + 1$) for **4** and 837 ($M^+ + 1$) for erythrosine.

Diacetyldihydrofluorescein Ester of Codeine. A solution of codeine (45 mg, 0.15 mmol), DCC (31 mg, 0.15 mmol), DADF (**1**, 62 mg, 0.14 mmol), 4-(dimethylamino)pyridine (10 mg) in dry CH_2Cl_2 (1 ml) was stirred at r.t. for 2 h.The mixture was worked up exactly as described above for the preparation of **6**, to afford a residue which was crystallized from ether/petroleum ether to afford the ester derivative (50 mg, 38%): m.p. 126°; $[\alpha]_D^{25} = -103°$ (c = 0.2, $CHCl_3$). IR: 1760, 1710 and 1620. ^{1}H-NMR: 1.60 (*s*, 3H, AcO); 2.28 (*s*, 3H, AcO); 1.96–3.48 (*m*, 6H, $3CH_2$); 2.44 (*s*, 3H, NMe); 3.56 (*s*, 3H, MeO); 5.28–5.84 (*m*, 5H, 5CH); 6.497.96 (*m*, 12H, 12 arom. H). CI-MS: 700 ($M^+ + 1$). Anal. calc. for $C_{42}H_{35}NO_9 \cdot \frac{1}{2}\ H_2O$ (706.75): C 71.37, H 5.13, N 1.98; found: C 71.17, H 5.52, N 1.86.

Diacetyldihydrofluorescein Ester of Quinine. A solution of quinine (33 mg, 0.09 mmol), DCC (21 mg, 0.10 mmol), DADF (**1**, 42 mg, 0.10 mmol), 4-(dimethylamino)pyridine (5 mg) in dry CH_2Cl_2 was stirred at r.t. for 2 h. The mixture was worked up as described for **6**, and the residue triturated with petroleum ether to afford the pure ester (30 mg, 46%): m.p. 110°; $[\alpha]_D^{25} = +32$ (c = 0.2, $CHCl_3$). IR: 1750, 1700 and 1600. ^{1}H-NMR: 1.68 (*s*, 3H, AcO); 2.28 (*s*, 3H, AcO); 1.92–3.60 (*m*, 10H, $5CH_2$); 3.94 (*s*, 3H, MeO); 4.96–6.04 (*m*, 5H, 5CH); 6.24–8.76 (*m*, 15H, 15 arom. H). CI-MS: 725 ($M^+ + 1$). Anal. calc. for $C_{44}H_{40}N_2O_8 \cdot H_2O$ (742.83): C 71.13, H 5.69, N 3.77; found: C 70.87, H 5.50, N 3.82.

Diacetyldihydrofluorescein Amide of Mescaline. A solution of mescaline (50 mg, 0.24 mmol), DCC (50 mg, 0.24 mol), DADF (**1**, 50 mg, 0.11 mmol), 4-(dimethylamino)pyridine (5 mg) was stirred at r.t. for 2 h. The mixture was worked up as described for **5**. The residue afforded on trituration with petroleum ether afforded the amide (40 mg, 28%): m.p. 94°. IR: 1750, 1650, 1600. ^{1}H-NMR: 1.56 (*s*, 3H, AcO): 2.28 (*s*, 3H, AcO); 2.92 (br. *s*, 4H, $2CH_2$); 3.80 (*s*, 9H, 3MeO); 5.80 (*s*, 1H, CH); 6.46–7.22 (*m*, 11H, 11 arom. H). CI-MS: 612 ($M^+ + 1$). Anal. calc. for $C_{35}H_{33}NO_9 \cdot H_2O$ (620.66): C 67.73, H 5.52, N 2.25; found: C 67.94, H 5.40, N 2.01.

REFERENCES

[1] *H.V. Liebig*, J. Prakt. Chem. *85*, 241 (1921).

[2] *A. Brossi*, Trends Pharmacol. Sci. *4*, 327 (1983).

[3] *H.G. Capraro & A. Brossi*, Tropolonic Colchicum Alkaloids, in 'The Alkaloids' Vol. XXIII, ed. A. Brossi, in press.

[4] Utilized in the synthesis of esters of Quinghasu-lactol; Prof. Song Zhen-yu, Institute of Materia Medica, Beijing, China. Personal communication.

[5] *C.F. Chignell*, Laboratory of Environmental Biophys., NIH, Research Triangle Park, N.C. personal communication.