Synthesis of Fluorescent 5-(2-Hydroxyaryl)-7-substituted-2,3-dihydro-1*H*-1,4-diazepines and Related Fluorescent 1,5-Benzodiazepines

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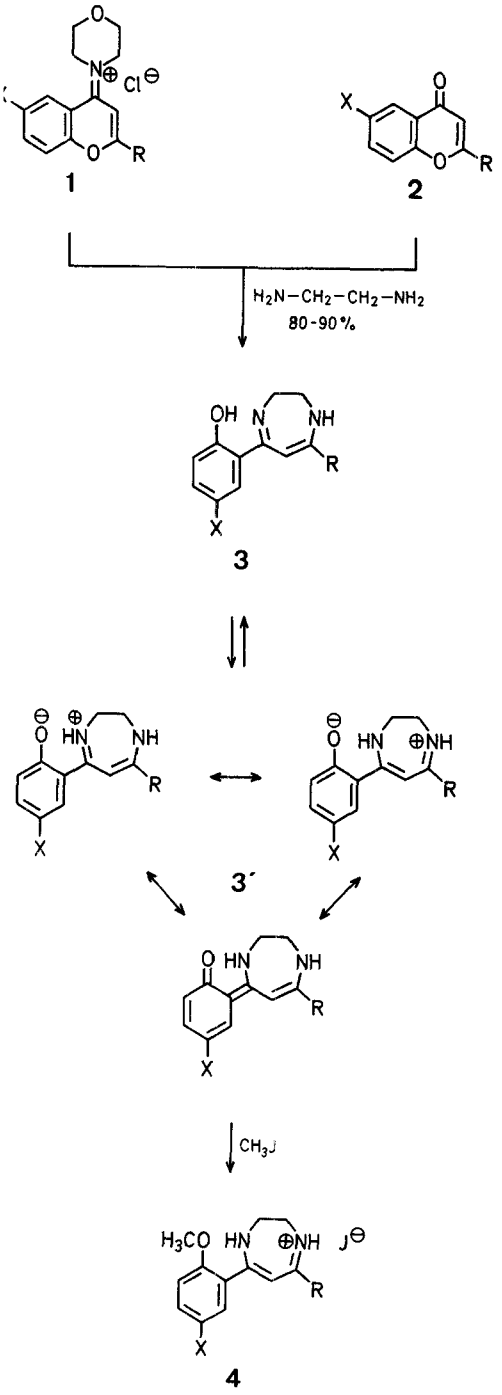
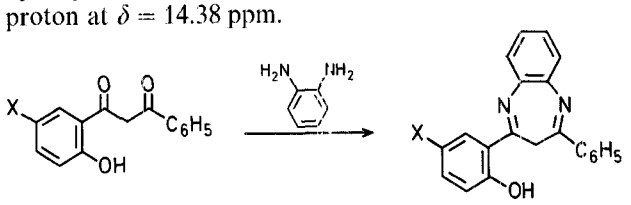
The present investigation evolved from earlier work on the reactive substance 4-morpholinoflavylium chloride (**1a**)[1]. This compound, as well as flavone (**2a**) and two of its derivatives **2b** and **2c** containing chloro or methyl substituents in the 6-position, and also certain chromones, **2d** and **2e**, having a heterocyclic substituent with aromatic character (2-furyl or 3-pyridyl) in the 2-position react readily with aqueous ethylenediamine solutions to yield 5-(2-hydroxy-



aryl)-7-substituted-2,3-dihydro-1*H*-1,4-diazepines **3** (Table 1). These bright yellow or orange products, some of which crystallize as hydrates, show when in the solid state, brilliant yellow-green, yellow or orange fluorescent colors under irradiation at 365 nm. The compounds have few of the expected characteristics of phenols and are markedly less basic than typical 2,3-dihydro-1,4-diazepines[2]. The structures of these products are apparent from their nuclear magnetic resonance spectra. Although surprisingly inert toward many acylating and reducing reagents, when treated with methyl iodide many of them are alkylated at the oxygen atom to yield methyl ethers **4**. This unexpected chemical behavior may be explicable on the basis of a tautomerism **3** ⇄ **3'**, which would lead to the presence of a species **3'** (three contributing forms shown) with some zwitterionic character and an increased nucleophilicity at the oxygen.

Benzodiazepines of related structure **(6)**, also yellow solids showing intense yellow fluorescence when irradiated at 365 nm, are obtained by condensation of the 1-(5-chloro- or 5-methyl-2-hydroxyphenyl)-3-phenyl-1,3-propanediones (**5a** or **5b**) with *o*-phenylenediamine, although reactions of **5a**, **5b** and similar 1,3-diketones with ethylenediamine give uncyclized products principally. A similar literature preparation of 2-(2-hydroxyphenyl)-4-phenyl-3*H*-1,5-benzodiazepine makes no mention of fluorescence[3]. Compounds of type **6** do not readily undergo methylation with methyl iodide. The proton nuclear magnetic resonance spectrum of **6a** indicates that the tautomeric form present in deuteriochloroform is that shown in the formula given; the diazepine ring contains a methylene group as indicated, and the phenolic hydroxy is hydrogen bonded, as indicated by the far downfield hydroxy proton at $\delta = 14.38$ ppm.



5a X = Cl
b X = CH_3

6a (64%)
b (78%)

When dissolved in organic solvents, compounds of types **3** or **6** show little or no fluorescence. However, fluorescence becomes evident in solutions made viscous as, for example, by the addition of glycerine, an effect which has been attributed in other instances to a slowing of certain intramolecular rotations which lead to internal losses of energy[4]. Solutions of these compounds in water-miscible organic solvents yield fluorescent mixtures when poured into aqueous solutions, apparently in some instances because of the separation from solution of tiny particles visible only by reason of their strong fluorescence. The presence of a small concentration of deoxyribonucleic acid (DNA) in an aqueous solution of **3a** nearly doubles the rather low fluorescent intensity otherwise observed (Table 2). The immediate effect of the prescence of DNA is smaller with **3b** and **6a**, but in the case of **6a** the presence of either DNA or bovine serum albumin delays over an extended period of time the separation of this water-insoluble benzodiazepine from initially rather strongly fluorescent ethanol/water mixtures. In the absence of DNA or the protein, complete separation of **6a** in the form of highly fluorescent particles occurs within a few hours. The behaviour of these compounds is in sharp contrast to that of the fluorescent dyes 8-anilinonaphthalene-1-sulphonate (ANS) and 2-(4-toluidinyl)-naphthalene-6-sulphonate (TNS), which, although showing little fluorescence in aqueous solution, give strongly fluorescent solutions in hydrophobic organic solvents[5]. Dilute solutions (1×10^{-3} molar) of benzodiazepines of type **6** in ethanol or tetrahydrofuran, when spotted on filter paper or silica gel thin layer plates, reveal no fluorescence initially, but the spots on paper are marked by an increasingly bright fluorescence (yellow) as the solvent evaporates. On the plates fluorescent rings appear after a time surrounding a dark center which will itself fluoresce strongly if a drop of water is introduced. Emission spectra corresponding to the visually observed yellow to orange fluorescent colors of the solid compounds of type **3** are obtained when the measurements are conducted on the materials in crystalline form. Thus, the fluorescent emission maximum for solid **3a** is at 522 nm (broad), that for **3b** at 560 nm (very broad). These maxima are at wave lengths 80 to 120 nm longer than those obtained from organic solvent/water mixtures containing the same compounds in solution (Table 2).

Table 2. Characteristics of the Fluorescence Observed in Ethanol/Water Mixtures and Powdered Crystalline Solids

Sample	Ethanol/Water Mixtures			Powdered Crystalline Solids	
	Excitation λ_{max} [nm]	Emission λ_{max} [nm]	Ratio FI/DNA to FI/Control	Excitation λ_{max} [nm]	Emission λ_{max} [nm]
3a	390	443	1.8	430 (est.)	522
3b	390	443	1.3	450 (est.)	560
6a	380	535	1.1	400	522

The substituted flavones **2b–e**[6,7] are prepared according to known procedures.

Table 1. 5-(2-Hydroxyaryl)-2,3-dihydro-1,4-diazepines **3** prepared

Product 3	X	R	m.p. [°C]	Yield [%]	Molecular formula[a]	U.V./Vis (ethanol) λ_{max} [nm] (log ε)
a	H		206–207°	86	$C_{17}H_{16}N_2O$ (264.3)	240 (4.16); 353 (4.00); 406 (4.24)
b	Cl		242–243°	84	$C_{17}H_{15}ClN_2O$ (298.8)	246 (4.39); 356 (4.04); 419 (4.24)
c	CH_3		197–198°	80	$C_{18}H_{18}N_2O$ (278.3)	243 (4.16); 355 (4.03); 419 (4.05)
d	CH_3		209–210°	90	$C_{16}H_{16}N_2O_2$ (268.3)	245 (3.81); 283 (4.01); 367 (4.09); 428 (4.15)
e	Cl		251–252.5°	85	$C_{15}H_{13}ClN_2O_2$ (288.7)	248 (4.15); 281 (3.96); 367 (4.04); 429 (4.23)
f	Cl		260.5–262.5°	90	$C_{16}H_{14}ClN_3O$ (299.7)	243 (4.28); 353 (3.99); 422 (4.16)

[a] Satisfactory microanalyses obtained: C ±0.17, H ±0.28, N ±0.18.

2-(2-Hydroxyphenyl)-7-phenyl-2,3-dihydro-1*H*-1,4-diazepine (3a):
A suspension of flavone (**2a**; 2.0 g, 0.009 mol) in 69.3% aqueous ethylenediamine (20 ml, 0.23 mol) is refluxed for 15 min. The resulting orange-red solution is cooled, diluted with glacial acetic acid (25 ml), poured over crushed ice, and allowed to warm to give a clear yellow solution, which is basified with 20% sodium hydroxide (~ 75 ml). The yellow solid which separates is collected by filtration and dried; yield: 2.053 g (86%); m.p. ~ 207 °C (dec.) Recrystallization from benzene gives yellow plates, m.p. 207.5–208.5 °C (dec.), showing a brilliant yellow-green fluorescence when irradiated at 365 nm.

The 1,4-diazepines **3b–e** are prepared by a slightly different method as illustrated below typically for **3b**.

5-(5-Chloro-2-hydroxyphenyl)-7-phenyl-2,3-dihydro-1*H*-1,4-diazepine (3b); Typical Procedure:
A solution of 6-chloroflavone (**2b**; 16.5 g, 0.064 mol) in 69.3% aqueous ethylenediamine (85 ml, 0.98 mol) is refluxed for 30 min, then diluted with water (40 ml), and allowed to cool to room temperature. Brilliant orange crystals separate out, which are recrystallized from benzene or absoluted ethanol; yield: 16.2 g (84%); m.p. 242–243 °C.

^{1}H-N.M.R. (CF_3COOH): $\delta = 4.09$ (br. s, 4H, —NH—CH_2—CH_2—NH—); 5.83 (br. s, 1H, =CH); 7.09 (d, 1H, $J = 9$ Hz, 3′-H_{arom}); 7.44 (dd, 1H, $J = 9$ Hz, 2.5 Hz, 4′-H_{arom}); 7.62 (m, 7H, 6′-$H_{arom} + C_6H_5$); 8.07, 8.60 ppm (2 br. s, 2H, —NH—CH_2—CH_2—NH—).

5-(2-Methoxyphenyl)-7-phenyl-2,3-dihydro-1*H*-1,4-diazepine (4a):
Hydroiodide of **4a**: A mixture of **3a** (2.0 g, 7.6 mmol) and methyl iodide (10 ml) is warmed briefly, then allowed to stand at room temperature in a stoppered flask. After 4 days the excess methyl iodide is removed by evaporation, and the solid residue is washed with anhydrous ether and crystallized from absolute ethanol at give the hydroiodide of **4a** as yellow cubes; yield: 2.39 g (70%); m.p. 184–185 °C.

$C_{18}H_{18}N_2OHJ$	calc.	C 53.21	H 4.71	
(406.3)	found	53.27	4.99	

I.R. (Nujol): ν = 3340, 3140, 2910, 2860, 1360, 1590, 1520, 1490, 1460, 1430, 1370, 1320, 1280, 1260, 1240, 1160, 1125, 1043, 1019 cm^{-1}.

Conversion of **4a** · HJ *to* **4a**: A solution of the hydroiodide (2.0 g, 4.9 mmol) in water (300 ml) is made alkaline with 5% sodium hydroxide solution (4.0 ml), the yellow solid precipitated is collected by filtration, washed with water, and dried. Recrystallization from petroleum ether (b.p. 65–110 °C) gives pale yellow crystals of **4a**; yield: 0.77 g (57%); m.p. 144–145 °C.

$C_{18}H_{18}N_2O$	calc.	C 77.66	H 6.52	N 10.10
(278.3)	found	77.22	6.74	10.07

5-(5-Chloro-2-methoxyphenyl)-7-phenyl-2,3-dihydro-1*H*-1,4-diazepine (4b):
A mixture of the dihydrodiazepine **3b** (8.0 g, 26.8 mmol) and methyl iodide (100 ml) is heated gently for 2 h under reflux. After removal of excess methyl iodide by distillation, the resulting yellow powder is dissolved in a minimum volume of hot ethanol and the solution is made alkaline with warm aqueous sodium hydroxide, then allowed to cool. The product which precipitates is recrystallized from benzene to afford deep-yellow fluorescent crystals; yield: 5.1g (62%); m.p. 223–224 °C.

$C_{18}H_{17}ClN_2O$	calc.	C 69.11	H 5.48	N 8.96
(313.5)	found	69.32	5.83	9.29

^{1}H-N.M.R. (CF_3COOH): $\delta = 3.98$ (s, 3H, OCH_3); 4.08 (br. s, 4H, —NH—CH_2—CH_2—NH—); 5.08 (br. s, 1H, =CH); 7.65 (m, 8H_{arom}); 8.06, 8.55 ppm (2 br. s, 1H each, —NH—CH_2—CH_2—NH—).

5-(5-Methyl-2-methoxyphenyl)-7-phenyl-2,3-dihydro-1*H*-1,4-diazepine (4c):
This is prepared analogously from **3c** and methyl iodide. Recrystallization from benzene gives fluorescent crystals; yield: 40%; m.p. 211–212 °C.

$C_{19}H_{20}N_2O$	calc.	C 78.05	H 6.90	N 9.58
(292.4)	found	77.95	6.82	9.68

I.R. (Nujol): ν = 3230, 2920, 1590, 1560, 1500, 1450, 1370, 1330, 1310, 1270, 1250, 1190, 1136, 1110 cm^{-1}.

2-(5-Chloro-2-hydroxyphenyl)-4-phenyl-3*H*-1,5-benzodiazepine (6a):
A mixture of 5-chloro-2-hydroxydibenzoylmethane (**5a**; 10.0 g, 0.036 mol) and *o*-phenylenediamine (10 g, 0.09 mol) absolute ethanol (100 ml) is heated under reflux for 3 h. Precipitation begins even before the mixture is cooled. The voluminous yellow product is recrystallized from absolute ethanol to give slender light-yellow needles; yield: 9.9 g (78%); m.p. 186–187 °C.

$C_{21}H_{15}ClN_2O$	calc.	C 72.72	H 4.36	N 8.08
(346.8)	found	72.58	4.63	8.18

^{1}H-N.M.R. ($CDCl_3$): $\delta = 3.75$ (s, 2H, CH_2); 6.85–8.15 (m, 12H_{arom}); 14.38 (s, 1H, OH).

2-(5-Methyl-2-hydroxyphenyl)-4-phenyl-3*H*-1,5-benzodiazepine (6b):
A mixture of **5b** (10.0 g, 0.039 mol) and *o*-phenylenediamine (14.5 g, 0.13 mol) in absolute ethanol (250 ml) containing acetic acid (0.8 drops) is refluxed for 20 h and cooled. The precipitate formed is collected by suction and recrystallized from absolute ethanol to give yellow fluorescent crystals; yield: 8.2 g (64%); m.p. 187–188 °C.

$C_{22}H_{18}N_2O$	calc.	C 80.95	H 5.56	N 8.58
(326.4)	found	80.81	5.67	8.34

U.V. (Ethanol): λ_{max} = 229 nm ($\log \varepsilon = 4.21$), 376 nm ($\log \varepsilon = 3.95$).

I.R. (Nujol): ν = 2900, 1610, 1590, 1560, 1530, 1490, 1460, 1370, 1330, 1310, 1290, 1250, 1240, 1210, 1200, 1150, 1110, 1072, 1052, 1090, 1020, 1000 cm^{-1}.

Fluorescence Measurements:
Measurements are made with a Hitachi-Perkin Elmer MPF-3 fluorescence spectrometer. Solutions in 95% ethanol of compounds **3a**, **3b**, and **6a** in the concentration range 1.5 to 2.5 mM are prepared. Addition of 30 µl quantities of each of these solutions to 3 ml of 10 mmolar Tris buffer (pH = 8.0) with rapid vortex mixing yields the fluorescent solutions to be placed in 3-ml cuvettes for the measurements. Effects of DNA or (in the case of **6a**) of bovine serum albumin are examined by adding to the 3-ml portions of Tris buffer 100 µl amounts of solutions containing one of these substances at a concentration of 10 mg/ml in 10 mmolar Tris buffer, thus giving final solutions in which the macromolecular additive is present at about 0.03% by weight. Fluorescent emission spectra from **3a** and **3b** in the crystalline state are measured on samples mounted in the beam after application in powdered form to small strips cut from a silica gel T.L.C. plate. The procedure yields well-defined values for emission maxima, but permits only a very rough estimation (est. in Table 2) of the excitation maxima. Characteristics of the fluorescence observed from the three compounds are tabulated below. In the last column the fluorescent intensity (FI) in the presence of DNA is compared to that observed in its absence.

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