Fluorescent Analogs of Insecticides and Synergists. Synthesis and Reactions of Active-Site-Directed Fluorescent Probes

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Fluorescent analogs of insecticides, synergists, and a series of enzyme inhibitors have been synthesized. Synthesis of designed fluorescent molecules to meet specific requirements is important in the application of fluorescence spectroscopy to research involving the mechanism and mode of action of insecticides and synergists *in vitro* and *in vivo*. The 5-dimethylaminonaphthalene-1-sulfonyl (dansyl) moiety imparts favorable fluorescence characteristics to fluorescent probe molecules. Fluorescent probes which successfully mimic the activity of insecticides extend other research methods.

The ultimate potentials from the use of fluorescence spectroscopy in enzyme research are dependent on synthesis of fluorescent molecules to meet specific requirements. Most insecticides are nonfluorescent or have unsuitable spectral properties. Many present applications of fluorescence in insecticide research involve conversion of insecticides to fluorescent products as an aid in analysis. Others use fluorogenic substrates in kinetic studies.

This research involved synthesis of fluorescent analogs of insecticides and synergists and active-site-directed enzyme inhibitors. Equilibrium inhibitors have a special importance when they act as fluorescent probes (Chen and Kernohan, 1967; Himel and Mayer, 1969, 1970b; Himel *et al.*, 1970). When a suitably designed fluorescent probe is used, spectroscopic detection of complex formation between the probe molecule (inhibitor) and the enzyme (or protein) is possible. This removes some of the limitations of enzyme methods which rely on chemical detection of substrate reactions. Deductive logic of kinetic methods can be supplemented by thermodynamic data from equilibria.

Fluorescent probes are molecules whose spectral properties (excitation, emission, and quantum yield) vary with their environment (Chen, 1967; Stryer, 1968; Edelman and McClure, 1968). The concept of a fluorescent probe stems from research of Weber (1952), Laurence (1952), and Weber and Laurence (1954). Fluorescent groups which have higher dipole moments in the excited state than in the ground state act as fluorescent probes (Stryer, 1968). In a polar environment, the more dipolar excited state interacts so as to align further the solvent dipoles. In a less polar environment, less excited state interaction occurs. Energy losses in excited state interactions are reflected by changes in the fluorescence emission ($Em\lambda_{max}$).

A fluorescent probe molecule acting as an equilibrium inhibitor is in dynamic equilibrium at the active site or an exo area. Many spectral properties undergo dynamic change as the probe molecule enters the complex and as it leaves the complex. Included are lifetime of fluorescence, quantum yield, emission λ_{max}, and energy transfer processes.

The fluorescent molecules reported here contain the 5-dimethylaminonaphthalene-1-sulfonyl (dansyl) moiety. Its use in fluorescent probes has been discussed (Himel and Mayer, 1970a,b; Himel *et al.*, 1970). Spectroscopy of the covalently bound dansyl moiety in proteins was studied by Weber (1952). Hartley and Massey (1956) studied the nonspecific covalent binding of dansyl chloride with chymotrypsin and chymotrypsinogen. Comparable sulfonyl fluorides appear to be specific for the active site of cholinesterase-type enzymes (Fahrney and Gold, 1963).

Baker (1967) has discussed the exo and active-site areas of enzymes and has shown the existence of considerable bulk tolerance in most enzymes. That fact allows synthesis of useful fluorescence into insecticide and synergist analogs and a series of designed enzyme inhibitors. In our research we have found that the nature and extent of energy transfer process are critical in evaluation of design parameters.

Synthesis data on an extensive series of compounds are described. Many are new compounds and all were synthesized to spectroscopic purity requirements. All compounds were tested for photolytic stability. Inhibitors of cholinesterase and microsomal enzyme systems are reported. Critical design parameters exist which determine the utility of candidate fluorescent probe molecules synthesized for specific research purposes.

Apparatus. Fluorescence spectra were run on a G.K. Turner Spectro 210. This absolute spectrofluorometer presents corrected emission spectra and excitation spectra at constant energy (Turner, 1964). A modified method of Nabb and Whitfield (1967) was used in the pH Stat Method for reaction of acetylcholine with cholinesterase enzymes and for comparative data on inhibition. The pH meter used was a Radiometer Model 26 equipped for automatic titration.

Procedure. The fluorescence well of the Spectro 210 was temperature controlled at 25° C. Water was double distilled from glass and found to have low intrinsic fluorescence. Enzymes used in fluorescence studies were dissolved in 0.05 *M* tris buffer. All fluorescence experiments were checked for photolytic degradation, photooxidation, or oxygen quenching. Bovine erythrocyte and serum cholinesterase (Sigma Chemical Co., St. Louis, Mo.) were used for AChE and ChE experiments. Concentrations of the enzyme were adjusted to give specific rates of hydrolysis of acetylcholine bromide (AChE 0.058 μmol/ml/min, ChE 0.045 μmol/ml/min). The enzyme solution was adjusted to pH 7.5 with 0.005 *N* NaOH, with stirring and while blanketed with N_2. Inhibitors were added at appropriate molar concentrations and incubated for 5 min, a time dictated by our interest in equilibrium inhibition. Acetylcholine bromide (0.5 ml) was added to give a concentration of 10 μ*M*. Rates of hydrolysis were determined as an average of three replicates. Matacil (3-methyl-4-dimethylaminophenyl-*N*-methylcarbamate) was obtained as analytically pure material from Chemagro Chemical Co. for comparison purposes and was used under comparable conditions.

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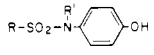
Reagents. Dansyl chloride (mp 68–70° C) was obtained from Peninsular Chemresearch. Solvents were reagent grade for synthesis and fluorescent grade for spectral studies. Pyridine was stored over solid KOH prior to use. Tlc film used to monitor synthesis reactions was Eastman, fluorescent, and nonfluorescent types.

Analysis. Microanalytical data are by Midwest Micro Laboratories. Structures of new compounds were confirmed by ir (Beckman IR-10) and nmr (Varian HA-100). In each case the spectra support the structure. We are indebted to D. R. Leyden for the nmr spectra. All syntheses were monitored by tlc and by ir.

Synthesis of Dansyl Phenolic Sulfonamides. PYRIDINE METHOD. Equimolar quantities (usually 0.004 mol) of the aminophenol (or salt) and dansyl chloride (usually 1.1 g) were added to 15 ml of dry pyridine. The reaction was kept under nitrogen and stirred over a 24-hr period. Ether and water were added and the ether layer was separated. The combined ether extracts were washed with 1% HCl and with water to remove traces of pyridine, dried over drierite, filtered, and ether was removed *in vacuo*. The reaction was monitored by tlc (benzene–acetic acid 75/25). Data are shown in Table I.

Synthesis of Dansyl Sulfonamides. Reactive amines give sulfonamides readily in ether, water, or aqueous acetone at room temperature. Aromatic amines were reacted in pyridine if necessary at elevated temperatures. Data on sulfonamides are shown in Table II.

Table I. Synthesis of Dansyl Phenolic Sulfonamides



Compd and no.	mp, °C	Solvent for recryst	Yield %	Analysis Calcd %	Analysis Found %	Comments
1 *p*-(5-Dimethylaminonaphthalene-1-sulfonamido)phenol	142–4	Benzene	62	C 63.15 H 5.26 N 8.18	63.00 5.28 8.05	Pyridine method using *p*-aminophenol hydrochloride
2 *p*-*N*-Methyl-(5-dimethylaminonaphthalene-1-sulfonamido)-phenol	114–5	Benzene/cyclohexane	45	C 64.04 H 5.62 N 7.86	63.70 5.86 7.79	Pyridine method using *p*-*N*-methylaminophenolsulfate
3 *m*-(5-Dimethylaminonaphthalene-1-sulfonamido)phenol	165–6	Benzene	50	C 63.15 H 5.26 N 8.18	63.27 5.41 8.16	Pyridine method using *m*-aminophenol

R = 5-(dimethylaminonaphthalene)-1-sulfonyl (dansyl); R′ = H or CH_3.

Table II. Synthesis of Dansyl Sulfonamides $RSO_2NR'R''$

Compd and no.	mp, °C	Solvent for Recryst	Solvent for tlc	Yield %	Analysis Calcd %	Analysis Found %	Comments
4 4-(5-Dimethylaminonaphthalene-1-sulfonamido)methylene-1,2-methylenedioxy benzene	91–3	EtOH–H_2O	Benzene	50	C 62.31 H 5.21 N 7.28	62.37 5.41 7.18	Pyridine method or $NaHCO_3$ method using piperonylamine
5 2-(5-Dimethylaminonaphthalene-1-sulfonamido)pyridine	235	EtOH	Benzene–MeOH 70:30	50	C 62.39 H 5.20 N 12.84	62.27 5.31 12.54	Pyridine method using 2-aminopyridine
6 3-(5-Dimethylaminonaphthalene-1-sulfonamido)pyridine	194	EtOH	Benzene–MeOH 70:30	50	C 62.39 H 5.20 N 12.84	62.39 5.22 12.89	Pyridine method using 3-aminopyridine
7 *N*-(5-Dimethylaminonaphthalene-1-sulfonamidoethylglycinate	76–7	EtOH–H_2O	Benzene–MeOH 70:30	50	C 57.14 H 5.95 N 8.33	56.77 5.90 8.23	Pyridine method using ethyl glycinate, *cf.* Deranleau and Neurath (1966)
8 *m*-(5-Dimethylaminonaphthalene-1-sulfonamido)benzotrifluoride	129–30	EtOH–H_2O	Benzene–MeOH 95:5	70	C 57.87 H 4.31 N 7.10	57.93 4.43 7.04	Pyridine method using *m*-aminobenzotrifluoride
9 *m*-(5-Dimethylaminonaphthalene-1-sulfonamido)ethylbenzoate			Benzene–MeOH 95:5	68	C 63.29 H 5.53 N 7.03	63.39 5.65 7.14	Pyridine method using *m*-aminoethyl benzoate. The compound is a solid with an indefinite melting point
10 *p*-(5-Dimethylaminonaphthalene-1-sulfonamido)benzoic acid	218–20	EtOH–H_2O	Benzene acetic acid 90:10	75	C 61.60 H 4.86 N 7.56	61.63 4.74 7.43	Pyridine method using *p*-aminobenzoic acid
11 *m*-(5-Dimethylaminonaphthalene-1-sulfonamido)benzoic acid	222–3	EtOH–H_2O	Benzene acetic acid 90:10	80	C 61.62 H 4.86 N 7.57	61.50 4.94 7.57	Pyridine method using *m*-aminobenzoic acid
12 5-Dimethylaminonaphthalene-1-sulfonamide	215	EtOH		100	*cf.* Weber (1952)		Prepared in this laboratory by Halim Aboul-Saad, using aqueous media [Weber (1952)]

(*Continued on next page*)

Table II. (Continued)

Compd and no.	mp, °C	Solvent for Recryst	Solvent for tlc	Yield %	Analysis Calcd %	Analysis Found %	Comments
13 *N*-Methyl-5-dimethylaminonaphthalene-1-sulfonamide	110–1	Benzene/cyclohexane	Benzene/cyclohexane/acetic acid 50:45:5	85	C 59.09 H 6.06 N 10.61	58.81 5.95 10.34	$NaHCO_3$–aqueous acetone method using methylamine hydrochloride
14 *N,N*-Dimethyl-5-dimethylaminonaphthalene-1-sulfonamide	100–2	MeOH–H_2O	Benzene/cyclohexane/acetic acid 50:45:5	85	C 60.43 H 6.47 N 10.07	60.53 6.62 9.86	$NaHCO_3$–aqueous acetone method using dimethylamine hydrochloride
15 (5-Dimethylaminonaphthalene-1-sulfonamido)benzene	141–2	EtOH–H_2O	Benzene	78	*cf.* Weber (1952)		Prepared in this laboratory by Halim Aboul-Saad, using aqueous media
16 *N*-Methyl(5-dimethylaminonaphthalene-1-sulfonamido)benzene	94–5	MeOH–H_2O	Benzene	78	C 67.06 H 5.88 N 8.24	66.57 5.94 8.18	Aqueous $NaHCO_3$–ether method using *N*-methylaniline. Prepared in this laboratory by Halim Aboul-Saad [Weber (1952)]
17 *N*-Benzyl-5-dimethylaminonaphthalene-1-sulfonamide	139	EtOH–H_2O			*cf.* Weber (1952)		Prepared in this laboratory by R. T. Mayer, aqueous method for reactive amines
18 8-(5-Dimethylaminonaphthalene-1-sulfonamido)quinoline	152–4	Benzene/cyclohexane	Benzene	58	C 66.84 H 5.04 N 11.14	66.61 5.17 11.22	Pyridine method using 8-aminoquinoline
19 8-(5-Dimethylaminonaphthalene-1-sulfonamido)-6-methoxy quinoline	207–8	Benzene	Benzene	73	C 64.86 H 5.16 N 10.32	64.87 5.21 10.39	Pyridine method using 8-amino-6-methoxy quinoline
20 *p*-(5-Dimethylaminonaphthalene-1-sulfonamido)benzyl diethyl phosphonate	162–3	EtOH–H_2O		78	C 57.98 H 6.09 N 5.88	57.19 6.10 5.90	Pyridine method using diethyl-*p*-aminobenzyl phosphonate, prepared by Halim Aboul-Saad

Table III. Synthesis of Dansyl Sulfonates from Phenols

RSO_2–OR′

Compd and no.	mp, °C	Solvent for Recryst	Solvent for tlc	Reaction time, days	Yield %	Analysis Calcd %	Analysis Found %	Comments
21 1,2-Dioxymethylene-4-(5-dimethylaminonaphthalene-1-sulfonato)benzene	86–7	Cyclohexane	Benzene	1	94	C 61.47 H 4.58 N 3.77	61.33 4.84 3.96	From sesamol [(4-hydroxy-1,2-dioxymethylene)]benzene
22 5-Dimethylaminonaphthalene-1-(4-nitrophenyl)-sulfonate	114	Cyclohexane	Benzene–cyclohexane acetic acid 50:45:5	1	63	C 58.07 H 4.30 N 7.53	58.37 4.41 7.47	From *p*-nitrophenol
23 5-Dimethylaminonaphthalene-1-sulfonato)-3-dimethylamino benzene	131–2	EtOH	Benzene	4	54	C 64.86 H 5.95 N 7.57	64.60 5.98 7.40	From *m*-dimethylaminophenol
24 4-(5-Dimethylaminonaphthalene-1-sulfonato)acetophenone	90–2	EtOH	Benzene–cyclohexane acetic acid 50:45:5	2	64	C 65.01 H 5.15 N 3.79	64.82 5.20 3.79	From *p*-hydroxyacetophenone
25 4-(5-Dimethylaminonaphthalene-1-sulfonato)acetophenone oxime	144–6	MeOH–H_2O	Benzene–cyclohexane acetic acid 50:45:5	1	40	C 62.48 H 5.21 N 7.29	62.48 5.32 7.24	Prepared from *p*-hydroxyacetophenone oxime

R = 5-(dimethylaminonaphthalene)-1-sulfonyl (dansyl); R′ = substituted phenols.

Table IV. Candidate Fluorescent Insecticides

Compd and no.	mp, °C	Solvent for Recryst	Solvent for tlc	Yield %	Elemental analysis Calcd %	Elemental analysis Found %	Comments
26 *p*-(5-Dimethylaminonaphthalene-1-sulfonamido)phenyl-*N*-methylcarbamate	160–2	Benzene	Benzene–acetone 5:1	67	C 60.15 H 5.26 N 10.52	60.49 5.55 10.28	Prepared from compound **1** in toluene. No catalyst
27 *p*-*N*-(*N'*-Methylcarbamoyl)-*N*-(5-dimethylaminonaphthalene-1-sulfonyl)phenyl-*N''*-methylcarbamate	159–61	Acetone petroleum ether	Benzene–acetone 5:1	97	C 57.89 H 5.26 N 12.28	57.82 5.47 12.00	Prepared from **1** using excess isocyanate and triethylamine as a catalyst
28 *p*-(5-Dimethylaminonaphthalene-1-sulfonato)acetophenone (*N*-methylcarbamoyl)oxime	114–5	Acetone–petroleum ether	Benzene–ethyl acetate 1:1	77	C 59.86 H 5.21 N 9.52	59.58 5.37 9.47	Prepared from compound **25**
29 *p*-(5-Dimethylaminonaphthalene-1-*N*-methylsulfonamido)phenyl-*N*-methylcarbamate	50 dec		Benzene–acetic acid 75:25	80			Prepared from **2**. This compound is photolytically unstable, hygroscopic. It is difficult to recrystallize and rigorously purify
30 5-Dimethylaminonaphthalene-1-sulfonyl fluoride	50–2	95% EtOH	Benzene–acetone 5:1	95	C 56.92 H 4.74 N 5.53	57.49 4.90 5.53	Prepared according to the method of Sigler *et al.* (1966)

Table V. Model Compounds

Compd and no.	mp, °C	Solvent for Recryst	Solvent for tlc	Yield %	Elemental analysis Calcd %	Elemental analysis Found %	Comments
31 *p*-Benzenesulfonamidophenol	157–8	Toluene	Benzene–ethyl acetate 75:25	88	*cf.* Tingle and Williams (1907)		Aqueous $NaHCO_3$ method at 0° C
32 *p*-Benzenesulfonamidophenyl-*N*-methylcarbamate	137–8	Acetone–H_2O	Benzene–ethyl acetate 75:25	100	C 54.90 H 4.58 N 9.15	54.87 4.58 8.93	Prepared by reaction of methyl isocyanate with compound above in anhydrous toluene or benzene. No catalyst
33 *p*-*N*-Methylbenzene sulfonamidophenol	135–6	Toluene	Benzene–acetic acid 75:25	81	C 59.32 H 4.94 N 5.32	59.48 4.98 5.23	Aqueous $NaHCO_3$ method at 0° C
34 *p*-*N*-Methylbenzene sulfonamidophenyl-*N*-methylcarbamate	115–7	Acetone–petroleum ether	Benzene–ethyl acetate 75:25	100	C 56.25 H 5.00 N 8.75	56.31 5.09 8.81	Prepared by reaction of CH_3NCO with compound above using triethylamine as a catalyst

Synthesis of Dansyl Sulfonates from Phenols. The phenol (0.02 mol) was added to dansyl chloride (0.02 mol) in 300 ml of ether containing 50 ml of triethylamine (free of dimethylamine). The reaction was monitored with tlc. Reaction times averaged 4 days. The ether solution was washed with water and then dried over drierite. The ether was removed *in vacuo*. The product was recrystallized as indicated. Data are given in Table III.

Synthesis of Fluorescent Insecticide Analogs. *N*-Methylcarbamates were prepared by reaction of methylisocyanate with dansyl phenolic sulfonamide (or oxime). Secondary sulfonamides containing a phenolic OH can react to give the carbamate or the ureacarbamate. The former is formed in the absence of catalyst in benzene or toluene solution. The latter is formed when triethylamine is used in catalytic quantities. Reaction times were 24 hr. Data are given in Table IV. Data on model compounds are given in Table V. Enzyme inhibition data are given in Table VI.

RESULTS AND DISCUSSION

Most dansyl derivatives exhibit useful fluorescent properties. Dansyl sulfonamides of reactive amines are readily prepared such that binding properties from differences in the sulfonamido substituent can be studied. Important relationships between binding characteristics and structure have been found in the series of compounds reported herein. The sulfonamido proton appears to have significant biochemical importance in enzyme and protein binding. The protolysis kinetics of the sulfonamido proton have been studied (Whidby *et al.*, 1971).

In order to prepare fluorescent phosphate and carbamate analogs of insecticides, pure sulfonamido phenols were required. The synthesis route was from the bifunctional aminophenol, reactive at both amino and phenolic groups. Prior to the development of tlc methods, specificity of reaction at the amino group was generally assumed, or byproduct sulfonate was removed or ignored. Most synthetic procedures in which dansyl chloride (**1**) was reacted with an aminophenol such as *p*-aminophenol gave a mixture of phenolic sulfonamide (**2**), aminosulfonate (**3**), and sulfonate-sulfonamide (**4**) (*cf.* Figure 1). The synthesis routes are simple but preparation of spectroscopic grade product was complex. The pyridine method allowed production of pure **2** directly, a unique and unexpected specificity of reaction.

Table VI. Enzyme Inhibition Studies on Bovine Erythrocyte Acetylcholinesterase (AChE) and Horse Serum Cholinesterase (ChE)

Compd and no.	Inhibition % of controls[a,b] 1×10^{-6} M AChE	1×10^{-6} M ChE	1×10^{-5} M AChE	1×10^{-5} M ChE	5×10^{-5} M AChE	5×10^{-5} M ChE	10×10^{-5} M AChE	10×10^{-5} M ChE	Comments
26 *p*-(5-Dimethylaminonaphthalene-1-sulfonamido)phenyl-*N*-methylcarbamate			3 ± 2	6 ± 2	10 ± 2	30 ± 2	14 ± 2	40 ± 4	10×10^{-5} M appeared to be the limit of solubility in our assay media
27 *p*-*N*-(*N'*-methylcarbamoyl)-*N*-(5-dimethylaminonaphthalene-1-sulfonyl)phenyl-*N''*-methylcarbamate			0.00	8 ± 5	6 ± 2	13 ± 3	11 ± 1	18 ± 1	*Ibid.*
28 *p*-(5-Dimethylaminonaphthalene-1-sulfonato)acetophenone (*N*-methylcarbamoyl)oxime			10 ± 1	33 ± 2	13 ± 1	43 ± 2	16 ± 1	47 ± 2	*Ibid.*
30 5-Dimethylaminonaphthalene-1-sulfonyl fluoride			9 ± 3	23 ± 1	18 ± 2	33 ± 3	24 ± 2	35 ± 2	
Matacil (4-dimethylamino-*m*-tolyl methylcarbamate)[c]	16 ± 5	10 ± 8	79 ± 2	30 ± 4		58 ± 3			Estimated I_{50}'s from experiment: I_{50} AChE = 4.7×10^{-6} M. I_{50} ChE = 3.6×10^{-5} M

[a] Average of three replicates. [b] Average activity of control for AChE = 10.71 μM of acetylcholine hydrolyzed per hour, and for ChE = 9.13 of acetylcholine hydrolyzed per hour. [c] Chemagro Corp., Kansas City, Mo.

Dansyl sulfonates from a variety of phenols were found to be reasonably chemically stable in water even at elevated temperatures. All sulfonates tested were, however, photolytically unstable to irradiation at the usual excitation wavelength in the range of 340 nm. Dansyl chloride (**1**) and dansyl fluoride are also photolytically unstable. Dansyl chloride is unstable in all solvents tested, and its fluorescence is quenched by the Cl to a major extent.

Dansyl fluoride is fluorescent in the same general range as other dansyl derivatives. It is an inhibitor of AChE and ChE. The inhibition of cholinesterase enzymes by sulfonyl fluorides has been studied by Fahrney and Gold (1963). Excellent evidence from X-ray diffraction studies indicates probable sulfonate formation at the active-site serine OH. Irradiation of the inhibited enzyme would be expected to give 5-dimethylaminonaphthalene-sulfonic acid (dans acid) λ_{max} 515 nm. Although dansyl fluoride and most dansyl sulfonates have little potential in *in vitro* spectroscopic studies, the ready production of dans acid on irradiation has excellent potentials in *in vivo* studies with fluorescence spectromicroscopy. The insecticidal properties of methanesulfonyl fluoride have been reported by Schrader (1952).

Photolytic stability is a critical design parameter for fluorescent probes. *p*-Substituted phenolic sulfonamides and their carbamate derivatives were less stable than the meta isomers but had acceptable stability. *tert*-Sulfonamides para to a phenolic OH were grossly unstable.

The fluorescent synergist analogs synthesized were found to be inhibitors of microsomal enzyme systems *in vitro* and *in vivo*. *N*-Alkyldansylsulfonamides vary in their ability to complex with serum albumin proteins. Equilibrium probes are useful in drug transport and insecticide transport studies (Jun *et al.*, 1971; Mayer and Himel, 1972). *N*-Aminoalkyldansylsulfonamide derivatives have been studied as equilibrium probes for the active site of ChE (Himel *et al.*, 1970; Mayer and Himel, 1972). Probes specific for the anionic site and the esteratic site are being studied.

The fluorescent carbamate (Figure 2) is a satisfactory structure for research purposes. It does inhibit AChE and ChE; its spectral characteristics are within the range outlined by Himel *et al.* (1970); and it has reasonable photolytic stability. Fluorescent probe characteristics are indicated by hypsochromic (blue) shift in the emission maximum from 565 to 520 nm when ChE is added and to 510 nm when AChE is added. It is reasonable to presume that the spectral shifts reflect equilibrium inhibition of the enzymes at the esteratic site since pH Stat data indicate inhibition (Table VI). Possible effects of exo area binding and extraneous protein binding are being studied. In the absence of extraneous protein binding these data represent spectral evidence for the existence of the carbamate–enzyme complex. The existence of the enzyme–substrate complex as the first step in reaction of enzymes was suggested by Michaelis and Menten as early as 1913. Kinetic proof of the existence of the insecticide–cholinesterase complex was obtained by Main (1964) and Main and Hastings (1966).

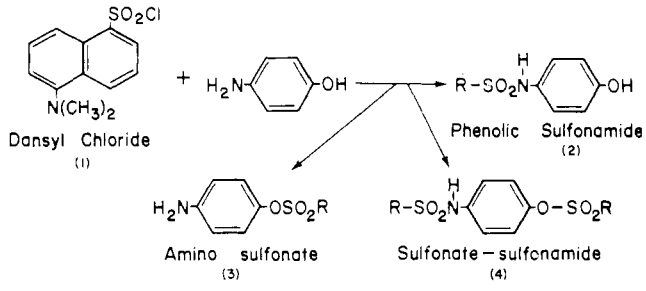


Figure 1. Synthesis route of dansyl chloride reacted with an aminophenol

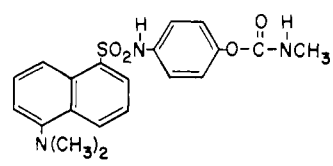


Figure 2. Fluorescent insecticide analog

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