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THE SEARCH FOR NEW BIOCHEMICAL PHOTOPROBES. II[1]. THE NUCLEOPHILIC PHOTOSUBSTITUTION OF 2-FLUORO-4-NITROANISOLE.

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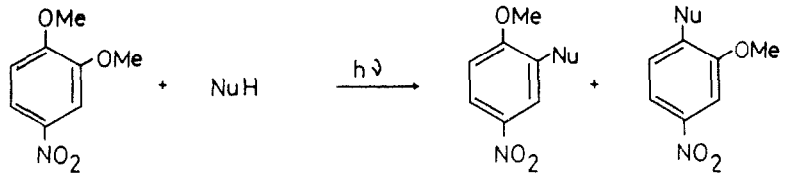
Abstract.- The photosubstitutions of 2-fluoro-4-nitroanisole with several amines are studied. The preparative results, the thermal stability of the photochemical substrate, and the limiting quantum yield values obtained from photoreactions with several nucleophiles suggest the possible usefulness of 2-fluoro-4-nitrophenyl ethers as biochemical photoprobes.

INTRODUCTION.-

The use of photochemically activated reagents in biology and medicine has gained big importance in the latest years[2]. They can be divided into (i) photolabelling (ii) photoaffinity labelling and (iii) photocrosslinking reagents, and are composed of some or all of the following parts: photoprobes, thermal probes, label, and a linker which may contain a cleavable group. Photolabelling reagents are used to attach a label (a tag) on biological macromolecules and are usually composed of a photoprobe and a label. Photoaffinity labelling reagents are used to identify and analyze receptors on biological macromolecules by photochemical attachement of a label to the target[3]. They are as a rule modified receptor substrates with an auxiliary group (the photoprobe)[4] or with their own structure slightly modified to become photoactive by itself[5]. Photocrosslinking reagents are used to study interactions between two or more biological macromolecules. They usually contain one thermal probe and one photoprobe (or two photoprobes) bound together by a linker ideally cleavable. Common and most important to the different parts of the commented reagents are the photoprobes. A number of photoprobes have been described some of which are even commercially available in the form of reactive derivatives. In practice, by far the most used have been arylazides[2,6,7], with diazo compounds as the second most used type[2,6,]. In a few cases ketones[2,9] or aryldiazonium salts[2,10] have been used. In an idealized version, the photoprobe should be thermally stable and the key intermediate created after photolysis would posses global reactivity. That is, its reaction with any chemical entity at the target site would be instantaneous and irreversible. None of the currently popular photoactivatable chemicals have demonstrated such thermal stability and global reactivity. On the contrary the bane of many photolabelling experiments is low efficiency of covalent bond formation after photolysis. Basic photochemical studies[11] have shown that even though it is frequently stated that arylazido photoprobes react via nitrene species this is often not the case. All evidence now indicate that the labelling species are azacyclo-

heptatetraene derivatives (which only react with nucleophilic functions) with the added drawback that only singlet excited azidoaryl photoprobes lead to covalent bonding to any large degree. Other recently proposed photoprobes, aroylazides[12], produce under irradiation long lived isocyanates as side products that will almost certainly cause unacceptable pseudolabelling. Most diazo compounds are thermally unstable even at room temperature and they are hydrolyzed in slightly acidic solutions. All this considered, there is an evident need to develop new thermally inert and globally reactive photoprobes.

The Nucleophilic Aromatic Photosubstitution has been the object of intense research for more than twenty years[13]. Cantor et al.[14] suggested the use of nitrophenyl ethers as photoprobes acting through S_NAr^* reactions (Scheme 1). Some examples[15] have been reported even though simple nitrophenyl ethers generally show relatively low efficiencies in photoreactions towards nucleophiles[16] and a certain propensity to photoreduce[17].



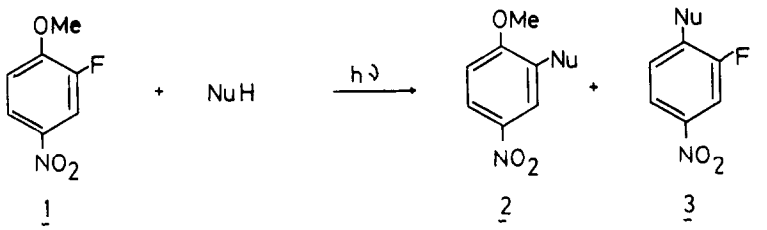
Scheme 1

The introduction of fluorine in a molecule normally alters its chemical and biological properties without dramatically altering its steric bulk[18]. These features make fluoro analogues of natural substrates interesting study subjects from the chemical and pharmacological point of view. Moreover, the introduction of fluorine in the proper position of a molecule can produce a photoactive new molecule. Nevertheless, and as far as we know, little is known about the photochemistry of polysubstituted fluoroaromatic compounds[19]. Following our research on new biochemical photoprobes[20] based on S_NAr^* reactions we have considered the possibilities of fluorine as a leaving group. Considering that an efficient photohydrolysis[21] had been reported for 2-fluoro-4-nitroanisole, 1, that the product is totally inert at room temperature in the normal photoreaction conditions and that it also shows a good thermal stability, we decided to study the reactions of product 1 with different amines, nucleophiles of particular relevance in biological chemistry. In the present paper we also present a comparative study of the efficiences of the photoreaction of 1 in front of different nucleophiles and the results are discussed from the viewpoint of their significance for the possible usefulness of 2-fluoro-4-nitrophenyl ethers as biological photoprobes.

RESULTS AND DISCUSSION.-

The photoreactions of 2-fluoro-4-nitroanisole, 1, with several amines are described in Scheme 2 and Table I. A 400W medium pressure Hg lamp was used in a Pyrex immersion well reactor. Irradiation was maintained until at least 80% of starting material had desappeared. Excess of amine was used in all cases. The photoreaction between product 1 and ethyl glycinate (exp.1, Table I) at pH 10 led to total consumption of starting material and the isolation of ethyl N-(2-methoxy-5-nitrophenyl)glycinate, 2a, in 43% preparative yield. 2-Methoxy-5-nitrophenol, 2g, was also obtained in 23% preparative yield. The total substitution yield amounted to 66%.

In similar conditions but at free pH and after only 1h of irradiation, the corresponding photoreaction between product 1 and methylamine (exp. 2, TableI) led to 68% total substitution yield distributed in: 40% N-methyl-2-methoxy-5-nitroaniline[22], 2b; 15% of N-methyl-2-fluoro-4-nitroaniline, 3b, characterized by elemental analysis and comparison of its spectral data with an independently prepared sample; and 13% of 2-methoxy-5-nitrophenol, 2g. All the yields are based on non recovered starting material (17% of 2-fluoro-4-nitroanisole, 1, was recovered after the photoreaction).



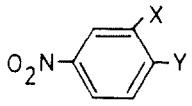
1 2 3

a) Nu: $-HNCH_2COOEt$
b) Nu: $-HNCH_3$
c) Nu: $-HNC_4H_9$-n
d) Nu: $-HNCHCOOEt$ with $(CH_2)_3CH_2NH_2$
e) Nu: $-HNC_6H_{13}$-n
f) Nu: $-N(CH_3)_2$
g) Nu: $-OH$
h) Nu: $-OCH_3$

Scheme 2

Experiment 3 (Table I) was performed in water/acetonitrile and led to similar results, being in this case the total photosubstitution yield 74%. The consumption of starting material in 4h time of photoreaction was 81% and the distribution of photosubstitution products was in this case: N-butyl-2-methoxy-5-nitroaniline[23], 2c, (26%), N-butyl-2-fluoro-4-nitroaniline, 3c, (19%), and 2-methoxy-5-nitrophenol, 2g, (29%). Products 2c and 3c gave correct elemental analyses and spectroscopic behaviour as expected.

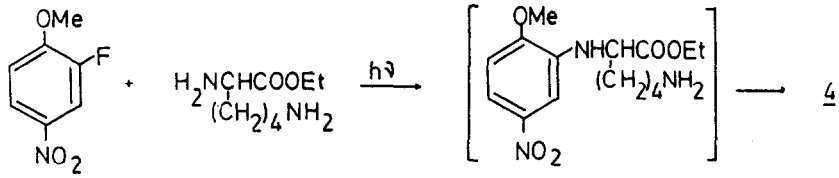
Table I.- Photoreactions of 2-fluoro-4-nitroanisole, 1, with amines.



Exp.	Amine	Reaction Conditions[a]	Reaction Products[b] X	Y		Yield(%)[b]	Total Yield(%)[b]
1	$H_2NCH_2CO_2Et$	H_2O/CH_3CN(70:30) pH 10, 7h	$HNCH_2CO_2Et$	OMe	2a	43	66
			OH	OMe	2g	23	
2	$MeNH_2$	H_2O/MeOH(80:20) 1h	HNMe	OMe	2b	40	68
			F	HNMe	3b	15	
			OH	OMe	2g	13	
3	n-$C_4H_9NH_2$	H_2O/CH_3CN(80:20) 4h	HNC_4H_9-n	OMe	2c	26	74
			F	HNC_4H_9-n	3c	19	
			OH	OMe	2g	29	
4	$H_2N(CH_2)_4CHCO_2Et$ NH_2	H_2O/CH_3CN(70:30) pH 10, 4h	$HNCHCONHCH_2(CH_2)_3$	OMe	4	6	28
			OH	OMe	2g	22	
5	n-$C_6H_{13}NH_2$	H_2O/MeOH(80:20) 1h	HNC_6H_{13}-n	OMe	2e	23	54
			F	HNC_6H_{13}-n	3e	5	
			OMe	OMe	2h	9	
			OH	OMe	2g	17	
6	Me_2NH	H_2O/MeOH(80:20) 4h	NMe_2	OMe	2f	6	69
			F	HNMe	3b	15	
			OMe	OMe	2h	12	
			OH	OMe	2g	36	

a) 400 W Medium pressure Hg lamp with pyrex filter. Room temperature. Excess of amine. b) Isolated yields with respect to non recovered starting material.

Photoreaction 4 (Table I) was somewhat more complicated. The presence of two amino groups in ethyl lysinate produced some problems. The photoreaction was less clean than the previously reported cases and even though the starting material was totally consumed we could only isolate a 6% yield of 2-(2-methoxy-5-nitrophenyl)amino-ε-caprolactam, 4 (Scheme 3) presumably resulting from cyclization of product 2d, the primary photosubstitution product of fluorine in 1 by the α-amino group of ethyl lysinate. Product 4 was characterized by elemental analysis and spectroscopic methods. Thus, the MS showed the molecular peak at 279 as the base peak, suggesting a very stable structure. The IR spectrum showed a strong amide band centred at 1655 cm^{-1}, and no ethoxy group could be observed in the ^1H NMR spectrum. All the spectroscopic evidence gave support to the proposed structure 4. This experiment suggests that 2-fluoro-4-nitroanisole, 1, prefers to react with the harder amino group of ethyl lysinate although the low yield obtained makes this conclusion tentative. This point will be discussed later. In addition to product 4, only 2-methoxy-5-nitrophenol, 2g, could be isolated in 22% yield.



Scheme 3

Experiment 5 (Table I) was carried out in water/methanol at free pH during only 1h of irradiation. After this time almost no starting material was left (3%) and the isolated product were: N-hexyl-2-methoxy-5-nitroaniline[16], 2e (23% yield), characterized by comparison with an authentical sample; N-hexyl-2-fluoro-4-nitroaniline, 3e, (5% yield) characterized by elemental analysis and comparison of the spectroscopic constants with those of an independently prepared sample; 4-nitroveratrole[24], 2h (9% yield); and 2-methoxy-5-nitrophenol, 2g (17% yield). The total photosubstitution yield was in this case 54%.

Photoreaction 6 (Table I) was performed in a similar way but the irradiation time was 4h. The total photosubstitution yield was in this case 69%. The starting material was consumed to an extent of 88%. The photoreaction was more complex than the preceeding case but two products could be isolated and two more were obtained as a mixture. The major photoproduct in this case was 2-methoxy-5-nitrophenol, 2g, (36% yield). The main amine photosubstitution product proved to be N-methyl-2-fluoro-4-nitroaniline, 3b, probably produced from N,N-dimethyl-2-fluoro-4-nitroaniline. Related cases in photochemical processes are reported in the literature[25]. In addition to those two products, a mixture of 4-nitroveratrole, 2h, and N,N-dimethyl-2-methoxy-5-nitroaniline, 2f, was isolated. The identification of the mixture components was performed by GC/MS which showed two peaks with molecular ions 183 (2h), and 196 (2f). The ^{1}H NMR spectrum of the mixture showed absorptions at 2.92 (s, NMe_2) and 3.9 (s, OMe), and the IR spectrum showed no N-H absorption.From the integration of the ^{1}H NMR signals yields of 6% and 12% for 2f and 2h could be deduced.

Remarkable features of these reported photoreactions are their high preparative photosubstitution yields and the low selectivity versus the nucleophiles present in solution. Thus, photohydrolysis is always present, and in the cases 5 and 6 (Table I) where the amine seems to be less reactive, even methoxide or methanol can act as nucleophiles. This low selectivity is considered a drawback when using these photoreactions in synthetic processes. Nevertheless it is clearly an advantage from the point of view of the bioorganic usefulness of 2-fluoro-4-nitrophenyl ethers as photoprobes. Another remarkable feature is the almost complete absence of photoreduction processes which are the main side photoreactions (or even the main process[17]) when other nitrophenyl ethers are used as photochemical substrates[26].

In Table II the limiting (in the presence of a large excess of nucleophile) quantum yields for the photoreactions of 2-fluoro-4-nitroanisole, 1, with different nucleophiles are

compared. Values for OH^- and H_2O were previously known and the others have been measured in the present work. The correspondig values for 4-nitroveratrole as a representative nitrophenyl ether are also included. The results of Table II confirm the relatively high and broad photoreactivity of 2-fluoro-4-nitroanisole, 1, when compared with other common photoprobes. We must remember here that 4-nitrophenylazide, one of the most widely used photoprobes in photolabelling experiments shows a very complex photoreactivity with amine nucleophiles and only a very small part of the excited 4-nitrophenylazide gives rise to a covalent bond with the amine nucleophile, photoreduction[11c] being the main process. This fact seems to be rather general for phenylazides. On the other hand the thermal inertness of product 1 gives to it clear advantages over diazo compounds[3]. The limitation of 2-fluoro-4-nitrophenyl ethers would be the necessity of the presence of nucleophilic functions (phenylazides have the same limitation) but most biological receptors with catalytic functions would present nucleophilic groups in their active sites. The observed photoreaction with neutral, relatively weak nucleophiles like H_2O and methanol is not a drawback for many applications since the environment in most cases is going to be lipophilic[10b]. On the contrary the viability of those photoreactions would ensure bond formation in a wide pH range.

From the presented results it can be concluded that 2-fluoro-4-nitrophenyl ethers can constitute a reasonable alternative to 4-nitrophenylazides and other previously published compounds as biochemical photoprobes.

Table II.- Limiting quantum yields for photosubstitution reactions of 2-fluoro-4-nitroanisole and 4-nitroveratrole with different nucleophiles.

Nucleophile[b]	Photosubstitution Quantum Yield[a] 2-fluoro-4-nitroanisole	4-nitroveratrole
OH^-	0.6[c]	0.09[d]
CH_3O^-	0.16[e]	0.23[f]
$n\text{-}C_6H_{13}NH_2$	0.07[g]	0.11[h]
H_2O	0.11[c]	$<10^{-3}$[i]
CH_3OH	0.005[i]	$<10^{-3}$[i]

a)In case more than one photosubstitution product appears, this value is the addition of the different quantum yields. b) Excess of nucleophile (no variation of the quantum yield with nucleophile concentration is observed at the used concentrations. c) Ref. 21. d) Ref. 27. e) This work, CH_3O^- =0.6M. f) Ref. 27. g) This work, $n\text{-}C_6H_{13}NH_2$ =0.6M. h) Ref. 16. i) This work. H_2O and CH_3OH were used as solvents.

EXPERIMENTAL PART.-

All melting points are uncorrected, ^1H NMR and ^{13}C NMR spectra were recorded at 80 and 20 MHz on a Brucker WP80SY spectrometer using TMS as internal standard. IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer or in a Nicolet ZDX FT-IR spectrometer. UV

spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. Mass spectra were recorded on a Hewlett-Packard 5985B mass spectrometer. The GC analyses were performed on a HP-5890A Gas Cromatograph using a HP-Crosslinked Dimethylsilicone Gum 12m x 0.2mm x 0.33m film thickness capillary column. Quantum yield measurements were performed on a Applied Photophysics QYR15 merry-go-round apparatus. The wavelenght of excitation was selected using a Jobin Ivon monocromator. 2-Fluoro-4-nitroanisole, 1, was prepared by nitration of 2-fluoroanisole following the procedure described in ref. 28.

Photoreaction between 1 and ethyl glycinate (Experiment 1, Table I). A mixture of 308 mg (1.8 mmole) of 1, 1.68 g of ethylglycinate hydrochloride (12 mmole), 150 ml of acetonitrile and 450 ml of a pH 10 (carbonate-bicarbonate) buffer solution was irradiated for 4 h in a Pyrex immersion well with a 400W medium pressure Hg lamp. The solvent was partially evaporated and the aqueous solution was extracted with methylene chloride. The organic layer was dried and evaporated to afford a residue (477 mg) which was chromatographed through 40 g of silica-gel using mixtures of hexane-methylene chloride as eluent. The obtained products were: Ethyl N-(2-methoxy-5-nitrophenyl)glycinate, 2a, mp 78-80°C (ether-pentane, Lit[29] 80-81°C), 0.195 g (35% yield), IR(KBr) 3400, 1730, 1515, 1335, 1200 cm^{-1}; ^1H NMR ($CDCl_3$) 1.31 (t, J=6.8 Hz, 3H), 3.39-4.79 (m, 10H), 6.75 (d, J=8.7 Hz, 1H), 7.28 (d, J=2.9 Hz, 1H), 7.67 (dd, J=8.7 Hz, J=2.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) 170.2, 151.8, 142.4, 137.4, 114.0, 108.2, 104.2, 61.5, 56.0, 45.1, 14.1; MS m/e(%) 254(M,17), 182(8), 181(100), 166(10), 165(8), 135(18). 2-Methoxy-4-nitrophenol, 2g, (0.073 g, 23%), mp 102°C (hexane, Lit[30] 102°C), IR(KBr) 3400, 1610, 1510,1340, 1280 cm^{-1}; ^1H NMR($CDCl_3$) 4.02 (s, 3H), 5.78 (s broad, 1H), 6.90 (d, J=9 Hz, 1H), 7.73-7.95 (m, 2H).

Photoreaction between 1 and methylamine (Experiment 2, Table I). A mixture of 308 mg (1.8 mmole) of 1, 12.330 g (180 mmole) of methylamine hydrochloride, 7.2 g (180 mmole) of sodium hydroxide, 120 ml of methanol and 480 ml of water was irradiated for 1 h in a pyrex immersion well with a 400w medium pressure Hg lamp. The solvent was partially evaporated and the aqueous solution was extracted with methylene chloride. The organic layer was dried and evaporated to afford a residue (260 mg) which was chromatographed through silica-gel using mixtures of hexane-methylene chloride as eluent. The following products were obtained: 2-Fluoro-4-nitroanisole, 1, recovered in 17% (52 mg). N-methyl-2-fluoro-4-nitroaniline, 3b, (38 mg, 15% yield based on non recovered starting material) mp.111-112°C (ether), IR(KBr) 3380, 1612, 1545, 1490, 1345 cm^{-1}; ^1H NMR($CDCl_3$) 3.00 (d, J=5.5 Hz, 3H), 4.5-5.0 (broad, 1H), 6.65 (dd, J=J'=9 Hz, 1H), 7.7-8.1 (m, 2H); ^{13}C NMR($CDCl_3$) 122.2, 122.1, 110.9, 109.7, 108.7, 108.5, 29.5; MS m/e (%) 170(M,4), 154(3), 140(100), 123(11), 112(32). Calculated for $C_7H_7FN_2O_2$: C, 49.41; H, 4.12; N, 16.47. Found: C, 49.30; H, 4.12; N, 16.67. N-methyl-2-methoxy-5-nitroaniline, 2b, (113 mg, 40% yield based on non recovered starting material) mp 85-87°C (Lit[22] 86-87.5°C) IR(KBr) 3200, 1600, 1540, 1520, 1340 cm^{-1}; ^1H NMR($CDCl_3$) 2.9 (d, J=6.5 Hz,3H), 3.95 (s, 3H), 4.4-4.6 (1H), 6.8 (d, J=9 Hz, 1H), 7.35 (d, J=2.5 Hz, 1H), 7.65 (dd, J=9 Hz, J=2.5 Hz, 1H). ^{13}C NMR($CDCl_3$) 151.6, 142.6, 139.6, 112.8, 107.7, 102.9, 55.9, 29.8. 2-Methoxy-5-nitrophenol, 2g, (33 mg, 13% yield based on non recovered starting material).

In order to completely characterize product 3b, it was synthetized in an independently way. Thus, a mixture of 855 mg (5 mmole) of 2-fluoro-4-nitroanisole, 1, 6.85 g (100 mmole) of methylamine hydrochloride, 4.0 g (100 mmole) of sodium hydroxide, 50 ml of methanol and 25 ml of water was heated to 90-95°C for 48 h under magnetic stirring in a stopped vessel. The solvent was partially evaporated and the aqueous solution extracted with chloroform. The organic layer was dried and evaporated to give a residue (784 mg) that by crystallization from ether afforded 705 mg (82% yield) of product 3b, mp 111-112°C.

Photoreaction between 1 and n-butylamine (Experiment 3, Table I). A mixture of 308 mg (1.8 mmole) of 1, 0.88 g of n-butyl amine (12 mmole), 100 ml of acetonitrile and 500 ml of water was irradiated for 4 h in a Pyrex immersion well with a 400W medium pressure Hg lamp. The solvent was partially evaporated and the aqueous solution was extracted with methylene chloride. The organic layer was dried and evaporated to afford a residue (412 mg). The aqueous layer was acidified and extracted with methylene chloride, giving rise to 73 mg (29% yield based on non recovered starting material) of 2-methoxy-5-nitrophenol, 2g. The first residue was chromatographed through silica-gel (60 g) using mixtures of hexane-ethyl acetate as eluent. The following products were obtained: N-Butyl-2-fluoro-4-nitroaniline, 3c, (59 mg, 19% yield over non recovered starting material), purified by destillation, bp 110°C(oven temperature/0.2 mmHg, IR($CHCl_3$) 3450, 1615, 1540, 1330 cm^{-1}; ^1H NMR($CDCl_3$) 0.81-1.93 (m, 7H), 3.26 (q, J=6.5 Hz, 2H), 4,5 (broad, 1H), 6.65 (dd, J=J'=9 Hz, 1H), 7.76-8.14 (m, 2H); MS m/e(%) 169(8), 123(10), 46(56), 43(46), 41(100), Chemical Ionization(isobutane) 213(M+1),

197(M-O +1), 183 (M-NO +1). Calculated for $C_{10}H_{13}FN_2O_2$: C, 56.60; H, 6.17; N, 13.20. Found: C, 57.18, 6.32, 12.85. N-Butyl-2-methoxy-5-nitroaniline, 2c, (85 mg, 26% yield based on non recovered starting material), purified by destilation, bp 120°C(oven temperature)/0.1 mmHg; IR(KBr) 3200, 1600, 1540, 1340 cm^{-1}; ^1H NMR($CDCl_3$) 1.0 (t, J=6 Hz, 3H), 1.2-1.8 (m, 4H), 3.2 (t, J=6 Hz, 2H), 3.9 (s, 3H), 6.8 (d, J=9 Hz, 1H), 7.35 (d, J=2.5 Hz, 1H), 7.7 (dd, J=9 Hz, J'=2.5 Hz, 1H); MS m/e(%) 224(M,17), 181(100), 166(12), 135(14). Calculated for $C_{11}H_{16}N_2O_3$: C, 58.91; H, 7.19; N, 12.49. Found: C, 58.93; H, 7.22; N, 12.37. And 2-fluoro-4-nitroanisole, 1, starting material (57 mg).

Photoreaction between 1 and ethyl lysinate (Experiment 4, Table I). A mixture of 308 mg (1.8 mmole) of 1, 2.96 g of ethyl lysinate dihydrochloride (12 mmole), 150 ml of acetonitrile and 450 ml of a pH 10 carbonate-bicarbonate buffer solution was irradiated for 4 h in a Pyrex inmersion well with a 400W medium pressure Hg lamp. The solvent was partially evaporated and the aqueous solution was extracted with methylene chloride. The organic layer was dried and evaporated to afford a residue (678 mg). The aqueous layer was acidified and extracted with methylene chloride, giving rise, after drying and evaporation, to 69 mg (22% yield) of 2-methoxy-5-nitrophenol, 2g. The first residue was chromatographed through silica-gel (60g) using mixtures of hexane-ethyl acetate-methanol as eluent. Only a defined product could be isolated, 2-(2-methoxy-5-nitrophenyl)amino-ε-caprolactame (32 mg, 6% yield), mp 194-5°C (ether-hexane), IR(KBr) 3415, 3250, 3110, 1655, 1620, 1525, 1330 cm^{-1}; ^1H NMR($CDCl_3$) 1.2-2.31 (m, 6H), 3.1-3.6 (m, 3H), 3.94 (s, 3H), 4.1 (d, J=9.8 Hz, 1H), 5.9 (broad, 1H), 6.77 (d, J=9.2 Hz, 1H), 7.15 (d, J=2.5 Hz, 1H), 7.63 (dd, J=9.2 Hz, J'=2.5 Hz, 1H); ^{13}C NMR($CDCl_3$) 175.7, 152.2, 145.5, 136.6, 113.2, 108.2, 103.3, 56.0, 55.1, 42.3, 30.5, 29.1, 27.9; MS m/e(%) 279(M,100), 262(10), 234(9), 217(34), 207(35), 179(43), 110(32), 84(81). Calculated for $C_{13}H_{17}N_3O_4$: C, 55.91; H, 6.14; N, 15.04. Found: C, 56.18; H, 6.14; N, 14.97.

Photoreaction between 1 and n-hexylamine (Experiment 5, Table I). A mixture of 308 mg (1.8 mmole) of 1, 3.64 g (36 mmole) of n-hexylamine, 120 ml of methanol and 480 ml of water was irradiated for 1 h in a pyrex inmersion well with a 400w medium pressure Hg lamp. The solvent was partially evaporated and the aqueous solution was extracted with methylene chloride. The organic layer was dried and evaporated to afford a residue (460 mg) which was chromatographed through silica-gel using mixtures of hexane-chloroform as eluent. The following products were obtained: N-Hexyl-2-fluoro-4-nitroaniline, 3e, as an oil (23 mg, 5% yield,), IR(film) 3405, 1615, 1545, 1330; ^1H NMR($CDCl_3$) 0.95 (distorted t, 3H), 1.1-1.9 (m, 8H), 3.25 (t, J=6.6 Hz, 2H), 4.5 (broad 1H), 6.64 (dd, J=J=8 Hz, 1H), 7.72-8.13 (m, 2H); ^{13}C NMR($CDCl_3$) 122.3, 122.2, 111.2, 110.0, 109.1, 108.9, 43.1, 31.4, 29.0, 26.5, 22.4, 13.8; MS m/e(%) 241(M+1,3), 240(M,17), 170(8), 169(100), 156(4), 123(32), 76(4). N-Hexyl-2-methoxy-5-nitroaniline, 2e, (102 mg, 23% yield) mp 34-36°C (Lit[16] 34-36°C), this product is completely described in ref 16. 4-Nitroveratrole, 2h, (30 mg, 9% yield) mp 95-97 (Lit[31] 98°C). And 2-Methoxy-5-nitrophenol, 2g, (50 mg, 16% yield).

Since no good elemental analysis for product 3e could be achieved and in order to confirm the propossed structure, it was synthesized by an independent way. Thus, a mixture of 308 mg (1.8 mmole) of 2-fluoro-4-nitroanisole, 1, 3.64 g (36 mmole) of n-hexylamine and 40 ml of methanol was heated to 120°C for 110 h under magnetic stirring in a stopped vessel. The solvent was partially evaporated and the aqueous solution extracted with chloroform. The organic layer was dried and evaporated giving a residue (428 mg) that after chromatography through acid alumina yields 138 mg (32% yield) of N-hexyl-2-fluoro-4-nitroaniline, 3e, as an oil. The spectroscopic constants were coincident with the previously reported for the product obtained from the photochemical reaction.

Photoreaction between 1 and dimethylamine (Experiment 6, Table I). A mixture of 308 mg (1.8 mmole) of 1, 4.92 g of 33% ethanolic solution of dimethylamine (1.62 g, 36 mmole), 120 ml of methanol and 480 ml of water was irradiated for 4.5 h in a pyrex inmersion well with a 400w medium pressure Hg lamp. The solvent was partially evaporated and the aqueous solution was extracted with methylene chloride. The organic layer was dried and evaporated to afford a residue (237 mg) which was chromatographed through silica-gel using hexane-methylene chloride as eluent. The following products were obtained: 2-Fluoro-4-nitroanisole, 1, starting material (40 mg). N-methyl-2-fluoro-4-nitroaniline, 3b, (34 mg, 15% based on non recovered starting material), mp 111-112°C (described in Experiment 2). A mixture (30 mg) 1:1 (based in ^1H NMR integration) of 4-nitroveratrole, 2h, identified by comparison (GC) with an authentic sample, and N,N-dimethyl-2-methoxy-5-nitroaniline[32], 2f, (aprox. 6% yield based on non recovered starting material) GC/MS m/e(%) 196(M,20), 181(29), 166(17), 149(9), 135(35), 123(11), 122(10), 120(12), 107(26), 106(29), 92(100). The ^1H NMR($CDCl_3$) of the

mixture showed absorbtions at 2.92 (s, NMe_2), 3.9(s, 2xOMe), intregrating 1:1 and a complex aromatic part. Finally 2-Methoxi-5-nitrophenol, 2g, (110 mg, 36% yield based on non recolvered starting material) was also isolated.

Quantum Yield measurements (Table II). Quantum yields for the photosubstitution products were measured using a merry-go-round apparatus. The irradiation source was a 250W medium pressure Hg lamp. The wavelength of excitation (366 nm) was selected using a monochromator. The amounts of photoproducts were determined by GC analyses (internal reference) as well as by UV absorbtion spectroscopy when possible. Actinometry was performed using potassium ferrioxalate[33], and conversion was kept around 5% in all the cases. Care was taken that >98% of the light were absorbed by the sample and the actinometer. No precautions were taken with the presence of oxygen. All the values are the result of five measurements eliminating the two extremes and averaging the other three.

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