PHOTOREACTION OF 3,4-DIMETHOXY-1-NITROBENZENE WITH BUTYLAMINE. A pH DEPENDENCE OF REGIOSELECTIVITY

Petr KUZMIČ, Libuše PAVLÍČKOVÁ, Jiří VELEK and Milan SOUČEK

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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Irradiation of 3,4-dimethoxy-1-nitrobenzene in the presence of butylamine leads to the formation of both possible photosubstitution products, *i.e.*, 2-methoxy-4-nitro-N-butylaniline and 2-methoxy-5-nitro-N-butylaniline with the predominance of the latter. Regioselectivity of the reaction as measured by molar ratio of the two isomeric products varies with pH of the solution, ranging from 3 : 1 at pH 10 to 12 : 1 at pH 12. The results are discussed in view of possible use of 3,4-dimethoxy-1-nitrobenzene moiety as a lysine-directed photoaffinity probe.

Photoaffinity labelling of biological molecules[1] is of great importance for studying biological interactions. Unfortunately, the most frequently used photogenerated reagents, nitrenes and carbenes, suffer from low selectivity which leads to undesired reactions with the solvent and/or to intramolecular reactions. Attempts were made to utilize the less reactive but more selective methoxynitrobenzene derivatives which react with amine nucleophiles yielding substituted nitroanilines. Jelenc and coworkers[2] have succeeded in photocrosslinking the γ-chains of human fetal hemoglobin using a bifunctional reagent containing the 4-nitroveratrole moiety. They showed in a model study that α-amino groups of amino acids as well as NH_2-termini of peptides are capable of forming the covalent crosslinks. Castello and coworkers[3] have synthetized a derivative of the same structural type as an intended photoreactive analogue of the antibiotic cycloheximide and have irradiated it in the presence of methylamine as a model nucleophile. The photosubstitution product was formed in reasonable yield.

In our laboratory we are currently interested in the design of an effective lysine-directed photoaffinity probe. On the basis of some preliminary results and literature data we reasoned that 3,4-dimethoxy-1-nitrobenzene represents a promising substrate for this purpose since it gives high preparative yields in photoreactions with amines. On the other hand, it has been demonstrated[4] that higher aliphatic amines exhibit considerable lack of regioselectivity in the photoreaction, yielding both possible substitution products. Thus, butylamine was reported[4] to react with the title compound with the formation of both *meta*- and *para*-nitroanilines in 21 and 15% yield, respectively. We tried to reproduce the earlier results under variety of condi-

tions but have found different regioselectivity than previously reported. Moreover, there seemed to be a dependence of regioselectivity on the pH of the medium. We therefore subjected the title photoreaction to a more detailed study the results of which we present in this paper.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. UV spectra were recorded in methanol on a Varian Cary 219 spectrophotometer, spectral band-width 1·5 nm. ^{1}H NMR spectra were obtained with a Varian XL-200 instrument in deuteriochloroform; chemical shifts are in δ units and the interaction constants in Hz. The assignment of aromatic protons is indicated by lower indices corresponding to the systematic numbering of the carbon skeleton. High-resolution mass spectra were measured with an AEI MS 902 spectrometer, electron energy 70 eV, source temperature 150—250°C. IR spectra were taken on a UR-20 (Zeiss) instrument in chloroform; positions of the absorption bands are given in cm^{-1}.

Flash-chromatographic separations were achieved on a column of silica gel (3 × 30 cm, particle size 30—60 μm, deactivated with 15% of water), elution with light petroleum–toluene 1 : 1 containing from 5 to 10% of acetone. Analytical high-performance liquid chromatography was performed on the modified silica gel Separon SI C-18 (Laboratorní přístroje, Praha), particle size 5 μm, column 25 × 0·4 cm, elution with methanol–water 7 : 3 v/v, flow rate 0·5 ml min^{-1}, UV detection at 365 nm. Preparative HPLC was run under the same conditions except of 20 μm sorbent particle size and 3·0 ml min^{-1} flow rate.

Tert-butanol and butylamine were distilled prior to use. Water was distilled from potassium permanganate. 3,4-Dimethoxy-1-nitrobenzene (*I*) was prepared according to the described procedure[5] and was crystallized twice from methanol, m.p. 96°C, ref.[5] m.p. 93—94°C. 2-Methoxy--5-nitrophenol (*IV*) was synthetized as reported by Drake and coworkers[6], m.p. 103—104°C, ref.[6] m.p. 104—104·5°C.

Preparation of 2-Methoxy-4-nitro-N-butylaniline (*III*)

3,4-Dimethoxy-1-nitrobenzene (*I*) (100 mg, 0·55 mmol) and butylamine (500 μl, 5·52 mmol) were mixed with 1·0 ml of 0·5M-NaOH and the mixture was heated at 120°C for 4 h in the dark. Excess of butylamine was evaporated *in vacuo*, the remaining semi-solid was dissolved in water (30 ml) and the solution was extracted with ether (3 × 10 ml). The collected ethereal extracts were dried with anhydrous magnesium sulphate, the solvent was evaporated *in vacuo* and the residue twice flash-chromatographed yielding the nitroaniline *III* as a yellow oil (71 mg, 57%). For $C_{11}H_{16}N_2O_3$ (224·3) calculated: 58·90% C, 7·20% H, 12·49% N; found: 58·81% C, 7·31% H, 12·35% N. ^{1}H NMR spectrum: 3·39 (s, OCH_3), 7·62 (d, $H_{(2)}$, $J_{2,6} = 2·4$), 7·91 (ddd, $H_{(6)}$, $J_{6,2} = 2·4$, $J_{6,5} = 9·0$, $J_{6,NH} = 0·6$), 6·49 (d, $H_{(5)}$, $J_{5,6} = 9·0$), 5·01 (bs, NH), 0·98 (t, $J = 7·2, 7·2$, CH_3), 1·45 (m, CH_2), 1·68 (m, CH_2), 3·24 (dt, $J = 7·0, 7·0, 5·6$, CH_2). UV spectrum: λ_{max} 230 nm (ε 610 m^2 mol^{-1}), 266 (460), 402 (1 360). IR spectrum: 3 435 (—NH—), 1 325, 1 539 ($—NO_2$).

Preparative Photolysis

3,4-Dimethoxy-1-nitrobenzene (*I*) (182 mg, 1·00 mmol) was dissolved in tert-butanol (200 ml) and the solution was mixed with 0·10M borate buffer of pH 11·0 (200 ml). Butylamine (1·50 ml, 15·7 mmol) was added, the solution was deoxygenated with the stream of argon (30 min) and subsequently irradiated in a preparative immersion-well photoreactor with a Pyrex-filtered

medium-pressure mercury lamp (RVK-125, Tesla) for 8 h while the stream of argon was continuously introduced. The reaction mixture was acidified with concentrated hydrochloric acid and saturated with sodium chloride. The aqueous layer was extracted with ether (3 × 50 ml), ethereal extracts were mixed with the tert-butanol layer, diluted with chloroform (200 ml) and dried with anhydrous magnesium sulphate. Solvent was removed by rotary evaporation and the residue subjected to flash-chromatography which yielded the unreacted *I* (125 mg), 2-methoxy-5-nitrophenol (*IV*, 6 mg, 11% based on the converted starting material) and a mixture (47 mg) of the nitroanilines *II* and *III*. An aliquot of the mixture (10·0 mg) was subjected to preparative HPLC (injection 1·0 mg per run) which gave 2·4 mg (16%) of *III* identified by comparison with the authentic sample and 7·6 mg (51%) of 2-methoxy-5-nitro-N-butylaniline (*II*) as a yellow oil. Elemental composition was confirmed by high-resolution mass spectrometry; for $C_{11}H_{16}N_2O_3$ calculated 224·1161; found 224·1156. ^{1}H NMR spectrum: 3·94 (s, OCH_3), 7·37 (d, $H_{(2)}$, $J_{2,6}$ = = 2·7), 7·61 (dd, $H_{(6)}$, $J_{6,2}$ = 2·7, $J_{6,5}$ = 8·8), 6·74 (d, $H_{(5)}$, $J_{5,6}$ = 8·8), 4·36 (bs, NH), 0·98 (t, J = 7·2, 7·2, CH_3), 1·46 (m, CH_2), 1·67 (m, CH_2), 3·18 (q, J = 6·5, 6·5, 6·5, CH_2). UV spectrum: λ_{max} 233 nm (ε 370 m^2 mol^{-1}), 263 (520), 307 (150), 400 (100). IR spectrum: 3 438 (—NH—), 1 343, 1 533 ($—NO_2$).

Determination of Regioselectivity

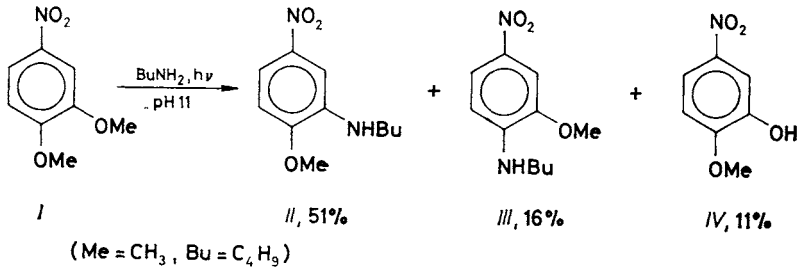
Butylamine (100 μl, 1·10 mmol) was injected into 20 ml of 0·10M-$Na_2B_4O_7$ in water and pH was adjusted to the desired value by 2·0M-NaOH. The resulting solution was mixed with 20 ml of the stock solution of aromatic substrate prepared by dissolving *I* (200 mg, 1·09 mmol) in acetonitrile (200 ml). The mixture was externally irradiated in a water-jacketed magnetically stirred cylindrical glass photoreactor (internal diameter 3·5 cm) at 25 ± 1°C. An Osram HBO-200 mercury lamp in a standard housing (Zeiss) with a condensor lens and a heat filter was used as a light source, distance 15 cm. Aliquots of the reaction mixture (20 μl) were taken off and were analyzed by HPLC; the irradiation was stopped at 15—20% conversion of the starting material. The respective total irradiation time varied in the range 10 min (pH 12·2) to 60 min (pH 10·0) and for higher pH was estimated from a conversion curve determined in advance. Acetonitrile was removed by rotary evaporation, aqueous layer was extracted with benzene (3 × 20 ml), organic extracts were dried with anhydrous magnesium sulphate and the solvent was evaporated *in vacuo*. The residue was redissolved in methanol (2·00 ml) and analyzed by HPLC. Molar ratio of the two isomeric photoproducts *II vs III* was determined from the integrated peak areas (elution volumes 18·6 ml and 14·4 ml, respectively) corrected to relative molar responses (1 : 5·35).

RESULTS AND DISCUSSION

In general accordance with the previously reported results[4], preparative photolysis of 3,4-dimethoxy-1-nitrobenzene (*I*) in aqueous tert-butanol at pH 11·0 and in the presence of butylamine yielded both isomeric nitroanilines *II* and *III* besides of a small amount of 2-methoxy-5-nitrophenol (*IV*), a product of the nucleophilic attack by hydroxide anion (see Scheme 1).

The total preparative yield of nitroanilines based on the converted starting material approaches 70%. In view of the possible use of 3,4-dimethoxy-1-nitrobenzene (*I*) as a photoaffinity label, the relatively high value of the preparative yield indicates reasonably low interference of side reactions. Moreover, some of the by-products, *e.g.* highly polar polymeric substances, can not be formed in a genuine photoaffinity

iabelling since in this case any interaction of two or more nitroaromate molecules ls excluded.



SCHEME 1

At constant analytical concentrations of both reactants, *i.e.* of butylamine and 3,4-dimethoxy-1-nitrobenzene (*I*), we varied the pH of the medium and registered the induced changes in regioselectivity as measured by molar ratio of the two isomeric photoproducts *II vs III* (see Fig. 1).

The *meta*-nitroaniline *II* is the main product in the whole range investigated here (pH 10·0–12·2) but it is apparent from Fig. 1 that there is a substantial drop of selectivity going to lower pH values. The loss of selectivity is also accompanied by a pronounced decrease of the overall quantum yield manifested by significant prolongation of the total irradiation time needed to achieve approximately the same conversion. On the other hand, HPLC analyses of the crude reaction mixtures showed that at lower pH the formation of the undesired photohydrolysis product *IV* is suppressed. We did not investigate the influence of OH^- ion concentration on the quantum yield and on the relative yield of *IV* in more detail. Let us only to

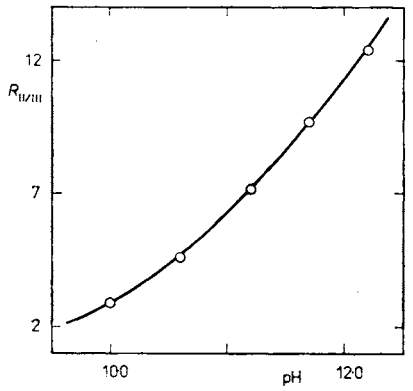
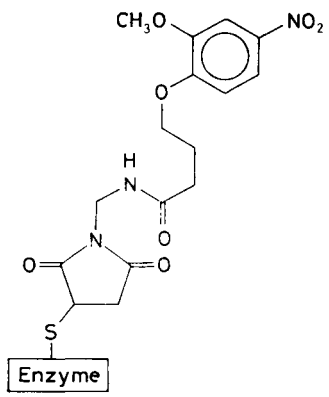


FIG. 1

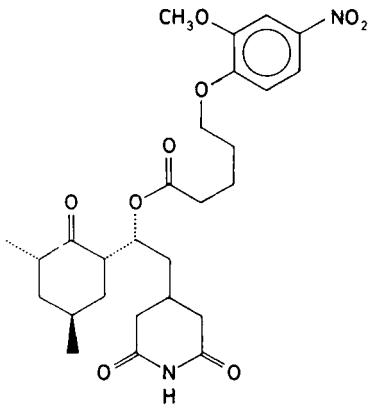
The molar ratio of isomers *II vs III*, $R_{II/III}$, formed in the photolysis of 3,4-dimethoxy-1-nitrobenzene (*I*, 2·22 mmol l^{-1}) and butylamine (22·5 mmol l^{-1}) in 50% aqueous acetonitrile in dependence on the pH

note that at least three factors seem to be operative which are difficult to assess separately. Firstly, the actual concentration of butylamine free base changes with the pH (pK_a 10·77). Secondly, the OH^- ions and methoxynitroaromatics are capable of forming triplet CT-exciplexes which, in some cases, were proved to be the genuine substrates for the attack of nucleophile[7]. Thirdly, hydroxide anion can also play the role of basic catalyst in the deprotonation step following the formation of the intermediate σ-complex[8,9].

We can conclude that 3,4-dimethoxy-1-nitrobenzene (*I*), when attached to biomolecules in the same manner as depicted in Scheme 2, would not be very advantageous as a lysine-directed photoaffinity probe despite of the relatively high preparative yield of the photoreaction with butylamine.



A (ref. 2)



B (ref. 3)

SCHEME 2

Indeed, in the media of lower alkalinity (pH 8–9) the use of derivative of the type 3-CH_3O-4-RCH_2O-$C_6H_3NO_2$ would lead to simultaneous label transfer and photocrosslinking (R is the handle connecting the biomolecule with the photolabel). Quite generally, the pH dependence of regioselectivity in the case of photoaffinity probes of the indicated design represents certainly a drawback, taking into account that local pH variations can be induced by microscopic environment.

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