SYNTHESIS AND PHOTOCHEMICAL REACTIONS OF NITROESTROGENS: POSSIBLE PHOTOAFFINITY LABELS OF ZERO LENGTH

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ABSTRACT

The syntheses of 3,4-dimethoxy-1,3,5(10)-estratrien-17-one and 4-bromo-3-methoxy-2-nitro-1,3,5(10)-estratrien-17-one are described and their photoreactions with amines and hydroxide ion studied. The possible usefulness of these new steroids as photoaffinity labels of zero length is discussed.

INTRODUCTION

Photoaffinity labeling is a useful method to identify biological receptor sites (1), aryl azides, diazocompounds, and aromatic ketones being the classical reagents used as photoactive moieties. Recently we have suggested (2,3) the use of 4-nitrocatechol ethers. These compounds are inert in the dark at room temperature, but under UV (ultraviolet) irradiation ($\lambda > 300$ nm) they react with nucleophiles by means of the so-called nucleophilic aromatic photosubstitutions which have been extensively studied by Havinga (4) and lately by other groups (5-11). In our laboratories (3) several biologically active molecules, among them the steroid estrone, have been modified incorporating a nitrocatechol ether as photoactive moiety. The basic photochemistry of the so modified

molecules in the presence of amines as nucleophiles has been also tested.

A common problem found following this general approach is the fact that the introduction of a relatively bulky photoactivatable moiety on a relatively small structure, such as a simple antibiotic or a steroid, can drastically modify the biological properties of the original substrate thus reducing its biological activity. An alternative and often better approach is the use of the intrinsic features of the biologically active molecule in such a way that it is used in photoaffinity labeling without or with only a slight modification (zero length label).

The physiologic properties of catechol estrogens and their methyl ethers are well known. Some examples of the use of nonmodified or slightly modified steroids as photoaffinity labels have been reported (12,13), and the presence of a functionalized aromatic ring in the catechol estrogens makes suitable the approach of zero length photoaffinity labeling through nucleophilic aromatic photosubstitution reactions.

We report here the synthesis of two new estrone derivatives with photoaffinity labeling potential, namely 3,4-dimethoxy-1-nitro-1,3,5(10)-estratrien-17-one, I, and 4-bromo-3-methoxy-2-nitro-1,3,5(10)-estratrien-17-one, II, and a preliminary study of the photoreactions of these new compounds with primary amines and hydroxide ion as models of biological nucleophiles.

EXPERIMENTAL

All melting points are uncorrected. ^{1}H-NMR and ^{13}C-NMR were recorded at 80 and 20 MHz on a Brucker WP80SY spectrometer using tetramethylsilane (TMS) as internal standard. Infrared (IR) spectra were recorded on a Perkin-Elmer 1310 spectrometer. Mass spectra were recorded on a Hewlett-Packard 5985B mass spectrometer. 3-Methoxy-1,3,5(10)-estratrien-17-one (estrone) was generously provided by Schering and Roussel-Uclaff companies. 3-Hydroxy-2-nitro-1,3,5(10)-estratrien-17-one (2-Nitroestrone, IV) (14-16), 3-hydroxy-4-nitro-1,3,5(10)-estratrien-17-one (4-nitroestrone, III) (14-16), 4-amino-3-hydroxy-1,3,5(10)-estratrien-17-one (4-aminoestrone, V) (17), 3,4-dihydroxy-1,3,5(10)-estratrien-17-one (4-hydroxyestrone, VI) (18), 2,4-dibromo-3-hydroxy-1,3,5(10)-estratrien-17-one (2,4-dibromoestrone, VIII) (19), 4-bromo-3-hydroxy-1,3,5(10)-estratrien-17-one (4-bromoestrone, IX) (20), 3-hydroxy-4-methoxy-1,3,5(10)-estratrien-17-one (4-methoxyestrone, X) (20) and 4-bromo-3-hydroxy-2-nitro-1,3,5(10)estratrien-17-one (4-bromo-2-nitroestrone, XI) (21) were prepared according to described procedures.

3,4-Dimethoxy-1,3,5(10)-estratrien-17-one, VII from 4-hydroxyestrone, VI

A mixture of VI (0.78 g, 2.7 mmol), K_2CO_3 (3.76 g, 21.6 mmol), dimethyl sulfate (1.33 g, 10.5 mmol), and butanone (140 mL) was heated at 60 C under stirring for 8 h. The mixture was filtered and the filtrate evaporated. The residue was chromatographed through a column of silica gel to afford 0.65 g (76%) of VII, mp 143-4 C; IR(KBr): 1725 cm^{-1}; ^{1}H-NMR($CDCl_3$): 0.91(s, 3H), 1.0-3.0(m, 15H), 3.84(s, 3H), 3.87(s, 3H), 6.74(d, J 9.1 Hz, 1H), 7.0(d, J 9.1 Hz, 1H); ^{13}C-NMR($CDCl_3$): 13.7, 21.5, 23.4, 25.9, 26.1, 31.6, 35.7, 37.8, 44.0, 47.8, 50.5, 55.7, 59.6, 110.1, 120.4, 130.7, 133.2, 146.6, 150.5, 218.5; MS: m/z 315(M+1, 18), 314(M, 100).

3,4-Dimethoxy-1-nitro-1,3,5(10)-estratrien-17-one, I

The diether VII (213 mg, 0.7 mmol) dissolved in the minimal amount of acetic acid was dropwise added to magnetically stirred fuming HNO_3 (0.1 mL of density 1.38 g/mL, 2.2 mmol) kept in an ice bath. The mixture was mantained in the ice bath for 1 h and poured into ice-water. The formed precipitate was filtered and dried to afford I (136 mg, 57%); mp 181-3 C (from ethanol); IR(KBr): 1720, 1520, 1360, 1340 cm^{-1}; ^{1}H-NMR($CDCl_3$): 0.95(s, 3H), 1.0-3.0(m, 15H), 3.94(two s, 6H), 7.21(s, 1H). Irradiation of the protons at 3.94 produced an Nuclear Overhauser Effect (NOE) effect on the absorption at 7.21; MS: m/z 359(M, 10), 343(24), 342(100), 115(31), 91(26), 77(30), 67(27), 55(57), 53(23), 43(21), 41(66). Calcd. for $C_{20}H_{25}NO_5$: C, 66.84; H, 7.01; N, 3.90. Found: C, 66.53; H, 7.14; N, 3.69.

4-Bromo-3-hydroxy-2-nitro-1,3,5(10)-estratrien-17-one, XI

A 5% (v/v) solution of bromine in acetic acid was added at 19 C over a stirred solution of IV (2.0 g, 6.3 mmol) in acetic acid (500 mL) and water (125 mL). Immediate decoloration was observed and the addition was continued until the red color remained. The mixture was poured on a mixture of ice-water (1 L). The formed precipitate was filtered off to afford XI (2.4 g, 96%). Recrystallization in acetone gave pure XI, mp 236-238 C, (Lit (21) mp 136-137 C, this datum is probably a misprint). $[\alpha]_D^{20}$ (in $CHCl_3$) = +109.2 (Lit.(21) $[\alpha]_D^{22}$ (in $CHCl_3$)= +110.1); IR(KBr): 3220, 1725, 1520, 1330 cm^{-1}; ^{1}H-NMR($CDCl_3$): 0.97(s, 3H), 1.0-3.0(m, 15H), 8.04(s, 1H); ^{13}C-NMR($CDCl_3$): 13.6, 21.4, 25.8, 26.1, 31.2, 31.8, 35.6, 37.1, 43.7, 47.5, 50.3, 115.5, 120.1, 131.9, 133.8, 147.9, 150.0, 219.2; MS: m/z 395(M+2, 100), 393(M, 99), 351(60), 349(60), 338(34), 336(43), 297(24), 295(25), 128(31), 115(50), 67(32), 55(38), 41(38).

4-Bromo-3-methoxy-2-nitro-1,3,5(10)-estratrien-17-one, II

A mixture of XI (0.5 g, 1.3 mmol), K_2CO_3 (0.9 g, 5.3 mmol), dimethyl sulfate (0.66 g, 5.3 mmol), and butanone (70 mL) was heated at 50 C under stirring for 1 h. The mixture was filtered and the filtrate was evaporated to afford 0.38g of II (73%): mp 136-7 C (from methanol); IR(KBr): 1725, 1510,1350 cm^{-1}; ^{1}H-NMR($CDCl_3$): 0.91(s, 3H), 1.0-3.0(m, 15H), 4.00(s, 3H), 7.78(s, 1H); ^{13}C-NMR($CDCl_3$: 13.6, 21.4, 25.8, 26.2, 31.3, 31.5, 35.6, 37.0, 44.1, 47.6, 50.3, 62.2, 120.9, 122.9, 138.1, 142.4, 143.8, 148.7, 219.4; MS: m/z 409(M+2, 81), 407(M, 84), 365(31), 363(33), 141(43), 129(33), 128(60), 115(100), 77(35), 67(61), 55(83), 41(84). Calcd. for $C_{19}H_{22}NO_4Br$: C, 55.92; H, 5.39; N, 3.43; Br, 19.58. Found: C, 55.98; H, 5.50; N, 3.60; Br, 19.54.

Photohydrolysis of II

6M NaOH (4.2 mL, 0.012 mol) was added to a mixture of II (0.154 g, 0.38 mmol), tetrahydrofuran (100 mL), and water (100 mL). The final solution was purged with argon and irradiated at room temperature under magnetic stirring with a 125-W medium pressure Hg lamp using Pyrex glassware. The reaction was monitored by UV/visible spectroscopy at 450 nm (absortion of the formed phenoxide ion). After 4 h the reddish solution was neutralized with 1 N HCl and most of the tetrahydrofuran evaporated. The resulting aqueous solution was extracted with chloroform. The organic layer was dried and evaporated. The residue was twice chromatographed through silica gel columns to afford recovered II (44%) and the phenol XI, mp 233-235 C (0.012 g, 8%).

A blank experiment in the absence of irradiation led to the recovery of II.

A similar photochemical experiment in the presence of

hexylamine led to the isolation of XI (5%) and to the recovery of II (61%)

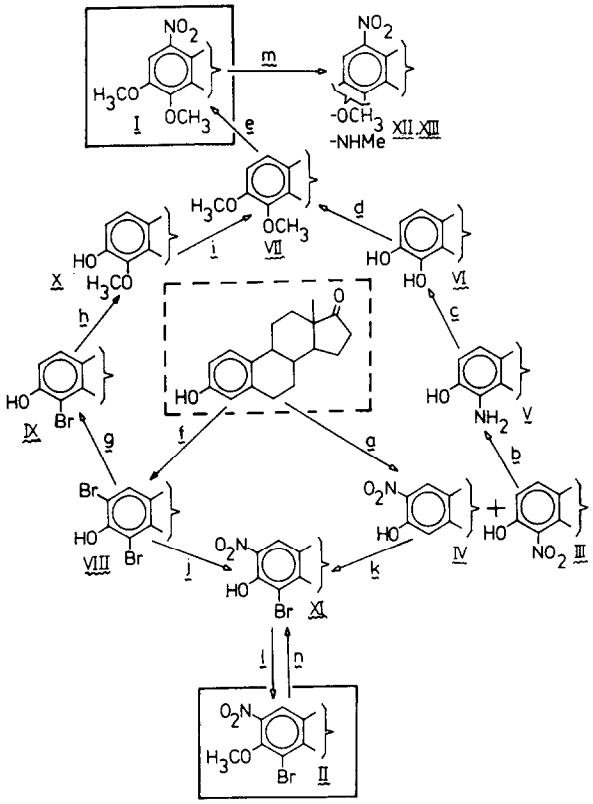
Irradiation of compound I in the presence of methylamine

A solution of compound I (147 mg, 0.4 mmol), methylamine hydrochloride (2.7 g, 40 mmol), and NaOH (1.6 g, 40 mmol) in a mixture of methanol (120 mL) and water (480 mL) was irradiated for 4 h at room temperature with a 400-W medium pressure Hg lamp using Pyrex glassware. The methanol was evaporated and the remaining mixture was partitioned between dichloromethane and water. The organic layer was dried and evaporated. The residue was filtered through a silica gel column to afford 40 mg of a mixture (gas liquid chromatography (GLC) monitoring), exhibiting UV absortion at $\lambda > 400$ nm, IR absortion at 3300 cm^{-1} and a peak in the mass spectrum (MS)at m/z 358(M^+). An GLC/MS analysis showed two different products with peaks at 358(M^+) and 341(M-OH), which can be attributed to the anilines XII and XIII. The main component of the mixture exhibited m/z 359(M^+) and was identified as I by comparison of the MS with an authentical sample.

A blank experiment in the absence of irradiation led to the recovery of I.

RESULTS

The first attempted synthetic route to compound I is represented in the scheme by the steps **a**, **b**, **c**, **d**, and **e**. Product I was obtained in 6.3% overall yield from estrone. The first step consisted in the nitration of estrone (14) that gives a 1:1 mixture of the 2- and 4-nitroisomers, only the 4-isomer being useful for our purposes. All the reported (15,16) improvements in the regioselectivity of this reaction lead to an increase of the 2-nitro/4-nitro isomers ratio. After conventional reduction (17) (step **b**) the amino group was transformed into a hydroxyl function (step **c**) through sodium periodate oxidation to ortho-quinone which without isolation was reduced with potassium iodide following the



a) HNO_3, AcOH, 45 C, 3 h. b) $Na_2S_2O_4$, NaOH, Acetone, H_2O, refl. c) **i**- $NaIO_4$, HCl aq., 30 min, room temp, **ii**- KI, AcOH, 2 min, **iii**- $NaHSO_3$ aq. d) Me_2SO_4, butanone, K_2CO_3, 60 C, 8 h. e) HNO_3, AcOH, 10 C, 1 h. f) Br_2, AcOH, 17 C. g) H_2, EtOH, Pd-C 5%. h) MeONa, DMF, $CuCl_2$, N_2. i) Same conditions as for (d). j) $NaNO_2$, AcOH, room temp, 30 min. k) Same conditions as for (f). l) Same conditions as for (d). m) hν, 400-W Hg medium pressure lamp, Pyrex filter (λ> 290 nm), $MeNH_2$ (excess), MeOH, H_2O, 4 h, room temp. n) hν , 125-W Hg medium pressure lamp, Pyrex filter (λ > 290 nm), NaOH, water, THF, 4 h, room temp.

SCHEME 1

procedure described by Staubenrauch and co-workers (18). However, this step cannot be easily scaled up. The transformation of VI into compound I was carried out by conventional methylation and nitration.

A second synthetic route to product I is described in the scheme by steps **f, g, h, i,** and **e.** This approach was intended mainly to overcome the difficulty of scaling up the first route, and gave a 4.3% overall yield of I from estrone. The dibromination of estrone is achieved in high yields using bromine in acetic acid (19). Compound VIII is an interesting starting material in the preparation of functionalized estrones. The path to compound X has been already described by Numazawa and co-workers (20).

The preparation of compound II is also shown in the scheme (steps **f, j,** and **l**). Product II is obtained in 38.5% overall yield from estrone. This product was also prepared in 26% overall yield from estrone by bromination of 2-nitroestrone (steps **a** and **k**).

Following our previous work (3,5) we have carried out a preliminary study on the photoreactions between the new estrone derivatives I and II and amines. Compound II was photoactive under UV ($\lambda > 300$ nm) irradiation (Hg medium pressure lamp, 4 h in tetrahydrofuran-water) in the presence of hexylamine. The photohydrolysis product XI (8% yield and 13% yield based on consumed II) was the only isolated photosubstitution product. This result was complemented by performing the photosubstitution of II

with sodium hydroxide in tetrahydrofuran-water, the hydroxyderivative I being now isolated in 8% yield (14% based on consumed II).

Compound I showed a different behavior. Its irradiation in the presence of methylamine led to a relatively complex reaction mixture. The formation of aromatic amines was inferred from the changes of the UV (appearance of absortion at 400 nm) and IR (appearance of a band at 3300 cm^{-1}) spectra. Analysis by GLC/MS of this reaction mixture showed several peaks, most of them of very low intensity and exhibiting molecular ions at low m/z values. Two main peaks will be considered: the more intense at shorter retention time corresponded to the starting material. The less intense at larger retention time was really formed by two overlapping peaks. Both showed the molecular ion at M^+ 358 arising from the substitution of a methoxy by a methylamino group and also important fragmentation signals at m/z 341 (M-17). This fragmentation of a OH moiety is characteristic of aromatic 1-nitrosteroids and in the starting nitroderivative I it gives rise to the base peak at m/e 342. This information supports the presence of a nitro group in the compounds of molecular weight 358. Considering all the discussed data these chromatographic overlapping peaks have been tentatively assigned to the isomeric N-methylanilines XII and XIII. The small amount of available

sample prevented any further characterization. No photohydrolysis products could be detected in the GLC/MS analysis of the mixture.

DISCUSSION

Recently (5) we have proposed a threefold mechanistic scheme to explain the photoreactivity of 4-nitroveratrole with amines and hydroxide ion. The three possible mechanistic pathways were bimolecular photosubstitution through the singlet excited state ($S_N2^1Ar^*$); the same but through the triplet state ($S_N2^3Ar^*$) and finally single electron transfer from the amine to the triplet excited state giving rise to a radical ion pair that by collapsing proceeds to the final products. The photosubstitution through the singlet excited state seems to require the previous formation of a donor-acceptor complex on the ground state. Models of the structures I and II show that the nitro groups cannot lay in the plane containing the aromatic ring. In compound I the nitro group interacts with the protons at C-11 whereas in compound II an important buttressing effect must operate due to the presence of nitro, methoxy, bromine, and alkyl substituents in consecutive carbon atoms. Moreover, the large bulk of these molecules makes the productive interaction between the very short lived singlet excited state and the nucleophile (in the $S_N2^1Ar^*$ mechanism) very unlikely. On the other hand, we have also reported (2,5) the extreme sensitivity of the single electron transfer mechanism to

the bulk of the substituents in the aromatic ring. Therefore, it is reasonable to assume that photosubstitutions on products I and II will proceed only through an $S_N2^3Ar^*$ mechanism. The alternative electron transfer to the triplet excited state would lead to photoreduction products (5).

The results here reported constitute an example of selectivity in front of different nucleophiles. Thus, the aromatic ring of product II is electronically poorer than that of compound I and it is selectively photohydrolyzed by hydroxide ion, a hard nucleophile with low lying frontier orbitals, even in the presence of hexylamine. On the other hand compound I photoreacts with methylamine, a softer nucleophile, and no phenols could be detected in the reaction mixture.

A general feature in the reported photoreactions is the low chemical yields. This might be originated by the reduced conjugation of the nitro groups on the aromatic rings and by the steric hindrance to the attack by the nucleophile.

There are obvious differences between the photoreactions here reported and the photolabeling of a biological macromolecule. The photoaffinity labeling agents interact with the receptor active site and are placed at bonding distance of the nucleophile. This situation is more favorable for photosubstitutions to occur than that encountered in the test photoreactions described in the present work. Therefore, the demonstration of the viability of the

photosubstitutions and the observed nucleophile selectivity makes this novel approach and the prepared products interesting as new tools in photoaffinity labeling.

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