CATALYTIC IODINATION AND DIRECT RADIOLABELLING OF DIHYDROTESTOSTERONE AND ESTRADIOL-17-DIPHOSPHATES

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Reaction involving iodine and cupric chloride has been applied to the synthesis of iodinated dihydrotestosterones while radiolabelled estradiol-17-diphosphates and dihydrotestosterone were prepared by the same path using $Na^{125}I$.

INTRODUCTION

In the last decade the androgen binding capacity of the prostate gland for testosterone, dihydrotestosterone, and their competitive antagonists was not only studied extensively but was also fully confirmed[1-3]. Therefore, these steroids when gamma labelled are expected to meet necessary criteria for imaging the prostate using external detection. In recent years Shida et al have reported few

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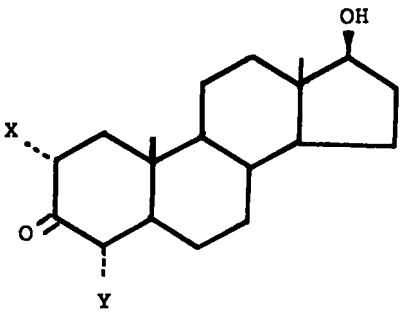
iodinated products (R_F values 0.60, 0.65, and 0.72, respectively) were made by comparison with data obtained on unlabelled analogs[5,6]. Total radioactivity yields up to 35% were achieved as yet. The relative distribution of compounds 2-4 was found to be 1:1:3. Similar ratio was reported for iodination of estradiol itself[9].

Iodination of biologically most active androgen, dihydrotestosterone, 5, was carried out at 60° during 1 h in chloroform-ethanol 1:1 and in the presence of $CuCl_2$, iodine, and few drops of HCl. After usual work-up procedure[9] the resulting product mixture was analyzed by TLC using benzene - ethyl acetate 1:1 as a solvent system. On developed chromatograms the spot comparating to 5 (2%) was observed (R_F 0.58) as well as two spots of reaction products (6, R_F 0.64, and 7, R_F 0.85). Compounds corresponding to 6 and 7 were isolated from a preparative silica TLC plate (89% and 3%, respectively) and recrystallized subsequently from ether-pentane (1:3). Monoiodo dihydrotestosterone, 6, mp 96-99°; mass spectral data, 416 M^+, 5%; UV (EtOH) λ_{max} = 256 nm (ε = 3960); IR (C=O) 1726 cm^{-1}; diiodo dihydrotestosterone, 7, mass spectral data, 542 M^+, 3%; IR (C=O) 1729 cm^{-1}. Negligible amount of products was formed both in the absence of proton source or by the addition of acetic acid.



	X	Y
5	H	H
6	I	H
7	I	I

of the reaction yield since the solvents used in TLC analysis leave some inorganic radioactivity[7] (ROH_2I, R_F 0.80) close to the spots corresponding to three radioiodinated products 2-4 (R_F values 0.60, 0.65, and 0.70, respectively[5]). It was found that ca 50% of the radioactivity has been associated with 2, 3, and 4 regardless of the reaction temperature (19°, 35°, and 45°). We believe that the positive iodine species are generated in a redox process involving iodine and cupric ion. The relative insensitivity of electrochemical reaction toward change in temperature[8] is in the line with results reported herein. This also implies the conversion of iodine to I^+ being the rate determining step. From the reaction mixture the inorganic radioactivity[7] corresponding presumably to $H_3O^+I^-$ (R_F value 0.05) was removed utilizing preparative TLC while radioiodinated products and ROH_2I complex were extracted from silica and eluted through an anion exchange column[6]. From the developed chromatograms of the eluate (Figure 1) structural assignements of radio-

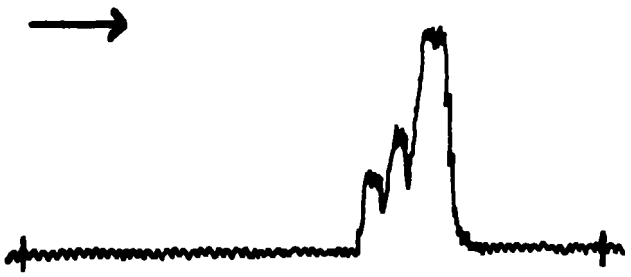


Figure 1. The radiochromatogram of radioiodinated estradiol-17-diphosphates obtained in this study

We assume that an electrophilic attack of I^+ on the enol form of 5 leads to the formation of products. The Δ^2-direction of the enolization in 3-keto steroids[10] and the superior stability of Δ^2- over Δ^3-cholestane[11] suggest the location of the iodine atom on C-2. Stereoelectronic control seems to prefer the formation of an axial iodo derivative[12]. In detailed studies on related 2-halo-3-ketones the 2α-bromo epimer was found as the sole product[13]. It appears that α-bromination is a consequence of both steric hindrance to the β-face attack on the enol and a "half-chair" conformation of ring A during electrophilic halogenation. Later conformational change of this ring into the chair form rationalizes the equatorial orientation of the carbon-halogen bond in the product[14]. Spectral data taken on 6 seem to parallel those obtained on 2α-iodocholestane[15]. Therefore, we tentatively assigned compound 6 as 2α-iododihydrotestosterone. This product was found to be reasonably stable after storing at -76° for 10 days.

Previously published results on the acid catalyzed rearrangement of 2,2-dibromo-3-keto steroids[16,17] to 2α,4α-dibromo derivatives can serve as indications in favor of diequatorial orientation of C-I bonds in 7. An intramolecular allylic rearrangement of the enol form of 2,2-diiodo derivative of 5 results presumably with its rapid isomerization to the more stable 2α,4α-diiododihydrotestosterone 7.

Compounds 6 and 7 were converted separately to their ^{125}I analogs by cuprous ion catalyzed exchange labelling method as described in the radioiodination of estradiol phosphate esters[6]. Radioactivity yields of ca 40% were determined radiochromatographically after 24 hours of the reaction course.

Direct labelling of 5 with $Na^{125}I$ was performed during 15 min at 65° in the manner described for chemical iodination of dihydrotestosterone and afforded exclusively more than 95% of radiolabelled carrier free 6 (R_F 0.64). This radioactivity yield drops to 35% in the absence of HCl as expected from the proposed reaction mechanism. In an effort to provide a clearer base for the potential use of radiotracer 6 in either RIA methods or in the prostate scintigraphy we found so far that this radiopharmacon is stable both at -20° in ethanol and at ambient temperature in the crude reaction mixture during 35 days.

CONCLUSION

The described synthetic procedure offers not only a suitable method for labelling the potential scanning agents for the prostate with short lived radioiodine but also opens the avenue for the preparation of other organotropic radioiodo pharmaca. Satisfactory radioactivity yield achieved in this study accompanied with the suitable reaction time seems to make the reported reaction an attractive alternative to other routes employed in radiopharmacy.

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7. In some supplemental studies we have observed that radiochromatograms of $CuCl_2$ - $Na^{125}I$ aqueous mixtures show one peak (R_F 0.05) when developed in aprotic and nonpolar solvents.The use of alcohols affords two peaks (R_F values 0.05 and 0.70-0.90). The location and intensities of these radioactivities depends on the acidity of the original mixture. Therefore, we believe that the first peak belongs to $H_3O^+I^-$ complex while the latter represents probably protonated alcohol molecule bound to iodine (ROH_2I).

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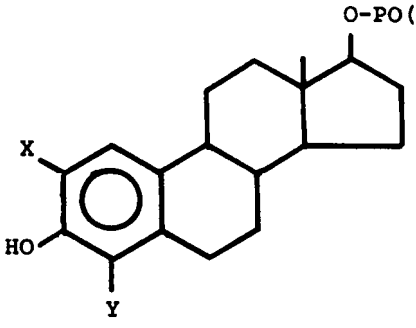
excellent scans of both human and rat prostate utilizing 2-^{131}I-estradiol-17-phosphate[4]. However, authors failed to furnish information about the synthesis of the compound applied. During our studies on the use of radiolabelled steroids for the visualization of the prostate we have prepared two radiomonoiodinated estradiol-17-diphosphates by exchange labelling methods[5,6]. In this progress report we describe the synthesis of the same compounds by direct labelling path as well as the preparation of radioiodinated dihydrotestosterone employing both direct radioiodination and exchange labelling procedures.

RESULTS AND DISCUSSION

Estradiol-17-diphosphate (1) was synthesized as reported previously[6]. The phosphate 1 was added to an ethanolic slurry containing anhydrous cupric chloride and Na^{125}I (The Radiochemical Centre, Amersham). From the reaction mixture aliquots were taken at several intervals of time and these samples were checked by radiochromatography using 1:1 ethanol-isopropanol as a solvent system. Simultaneously, control experiments were carried out without the substrate added. These runs were critical for the preliminary estimation



	X	Y
1	H	H
2	^{125}I	H
3	H	^{125}I
4	^{125}I	^{125}I