RADIOIODINATION OF IODINATED ESTRADIOL-17-DIPHOSPHATES[1]

M. Tarle, R. Padovan[2], and Š. Spaventi

Clinic of Nuclear Medicine and Oncology, Clinical Hospital "Dr.M.Stojanović", 41001 Zagreb, Croatia, Yugoslavia

Received August 16, 1977

KEY WORDS: monoiodoestradiol-17-diphosphates, radioiodine exchange labeling

Estradiol and its mono and diiodo derivatives were converted to their 17-diphosphate analogs. Radioiodine labeling of monoiodinated estradiol diphosphates with ^{125}I proceeds smoothly in cuprous chloride catalyzed exchange reaction utilizing acetonitrile-ethanol (1:1) as a solvent. Structural assignments of unlabeled compounds were made by means of mass spectral data, elemental analysis, and qualitative tests. Radiochromatographic analysis furnished information on the identity of radioiodinated products. Labeling yields up to 35-40% were achieved. Two possible mechanistic interpretations of cuprous ion catalyzed exchange labeling are proposed and their relative merits discussed. The results described in this paper seem to prefer, in harmony with arguments put forward in some previous studies, the exchange labeling *via* a four center transition state over an intervening $S_{NR}1$ type reaction.

INTRODUCTION

Over the past decade much effort has been made on the potential use of radionuclides for scanning the prostate. Despite the rapid development of numerous radiopharmaceuticals in nuclear medicine, the

0362-4803/78/0015-0007$01.00

prostate still remains one of few glands to be routinely visualized using external counting methods. A radiolabeled drug that selectively concentrates in the prostate can not be only a predictor of tumor response to hormone therapy but may also be used in an early detection of some disorders.

Repeated suggestions have been made that some radiolabeled steroids or compounds having steroidomimetic activity can meet necessary criteria for imaging of the gland examined. In recent years a number of workers have reported ample evidences for the accumulation of both estradiol and androgens in the prostatic gland. These results were based on either competitive binding assays[3,4,5] or by studying the radioactive uptake of tritiated analogs of these steroids after the in vivo injection[6,7,8]. However, data obtained when radioiodinated steroids were used for clinical purposes have been somewhat controversial. Tubis et al synthesized the radioiodinated diethylstilbestrol diphosphate by I-Cl addition to the central double bond[9a]. Unfortunately, when injected intravenously this material failed to produce a significant concentration in the prostate[9b]. Few years later Szendroi et al reported a prostate scan using again radioiodinated diethylstilbestrol diphosphate[10]. This time the agent was prepared utilizing modified Rosa's electrochemical method[11] which assures aromatic iodination of the substrate. The critical conformational differences between the compound that Tubis had dismissed and the agent used by Szendroi might account for rather discouraging results obtained in former studies[9b]. Waters et al published the results of the distribution studies of radioiodinated estradiol[12a,b] and 2α-^{77}Br-5α-dihydrotestosterone[13] in the rat and in the human prostate. These results lead to the conclusion that both compounds are not appropriate for scanning the prostate principaly due to the release of radionuclides in vivo. Finaly, Shida et al had used 2-^{131}I-estradiol-17β, 17-phosphate and reported a clear picture of both rat and human prostate gland[14]. Although the actual uptake

figures were rather low the sufficient retention time of the steriod administrated allowed the performance of the scintigraphic image of the gland. Unfortunately,the synthesis of the compound used was not described in this paper.

In previous studies we have synthesized three iodinated estradiols[15] showing that electrochemical preparation of iodinated estradiols, diethylstilbestrols[15],and diethylstilbestrol diphosphates[16] is preferable to other synthetic routes when the constant potential electrolysis is used as a technique of choice. The preparation of radioiodinated estradiols and diethylstilbestrols was achieved by exchange labeling in the presence of CuCl [17]. Binding affinity of 2-iodoestradiol and 4-iodoestradiol to specific receptors in mammary cytosol fraction has been determined[18]. A 200-fold greater concentration of 2-^{125}I-estradiol suppresses totaly the binding of the parent compound to specific macromolecules corresponding to 8S fraction while the 4-radioiodoestradiol shows only 80% of this activity. These results concerning the binding of two iodostereoisomers of estradiol in a target tissue correlate well with their partition coefficients in an octanol-water system[19a,b]. The iodine substitution at the C-4 position causes,in our opinion,a planarity distortion of the ring A [19a], thus probably lowering the affinity of the steroid to both sex steroid binding plasma protein (SBP)[20,21] and to specific receptors. Finally,the presence of the 17β-OH function in steroids is one of the critical parameters for their binding to androgen receptors[5].

Bearing all these points in mind it is hard to rationalize markedly different results for the <u>in vivo</u> uptake of radioiodinated estradiols[12] and their phosphate analog[14] without taking sharp differences in their hydrophilic properties into consideration[1]. Some insight in the characteristics of the possible mechanism of bioaction might be gained primarily by the <u>in vitro</u> determination of the binding properties of iodinated estradiol phosphate esters followed by

a rafined analysis of structure-activity relationship of these compounds,and by the steroid metabolism studies. Moreover,according to recent discoveries[22] the role of tyroxine-binding protein in binding to radioiodinated steroids has to be extensively studied. The present work summarizes the first part of the program which is instituted to investigate a reliable group of compounds that can be used as tumor localizing agents in diagnosis of prostate disorders,but may also be significant in tumor therapy.

EXPERIMENTAL

General. Commercial 17β-estradiol was reagent grade (Sigma,St. Louis,Mo.) and was used without further purification. Carrier free $Na^{125}I$ (for protein iodination) was purchased from The Radiochemical Centre,Amersham. All chemicals used in the synthesis and chromatographic analyses were reagent grade. Analytical thin layer chromatography (TLC) was performed on 10 x 4 cm plates uniformly covered with silica gel (0.25 mm, Silica Gel $H_{254+366}$ acc. Stahl, Merck,Darmstadt). Samples were spotted with a micropipette and chromatograms were developed in equilibrated tanks at room temperature to a height of 8 cm. Spots were visualized with a UV lamp or by spraying chromatograms with 50% aq. sulfuric acid. Compounds were located on developed chromatograms using an Actigraph III (Nuclear Chicago,Ill.) and an argon-ethanol mixture when the purity and identity of radioiodinated products was checked. A silica on a radiochromatogram corresponding to radioactivity was removed from the plate and was transferred to a counting vial. The actual radioactivity of spots was counted in an 1185 Automatic Gamma Counting System (Nuclear Chicago,Ill.). Ion exchange chromatography was performed using an anion exchange resin (Amberlite IRA-400-Cl,Rohm and Haas Co.,Pe.). The mass spectral data were recorded on a Varian CH-7 spectrometer. The temperature of the ion source was held at

40° and the sample temperature was held at 150°. An accelerating voltage of 1.7 kV was applied in several consecutive measurements. Melting points were determined on a Kofler block and are uncorrected.

Preparation and purification of 4-iodiestradiol (2), 2-iodiestradiol (3), and 2,4-diiodoestradiol (4). The mixture of iodinated estradiols was prepared using the chemical procedure as described in the previous publication[15]. Crude products were analyzed by TLC using several solvent systems. Methylene chloride as a solvent led to satisfactory separation of the unreacted starting material from iodinated derivatives on a preparative TLC plate. Further separation of iodinated estradiols was achieved using chloroform and acetone (9:1) as a solvent system in a single or double development manner (Table 1). Several repurifications (2-3) were required to obtain satisfactory purity of iodinated estradiols.

Preparation and purification of estradiol-17-diphosphate (5), 2-iodoestradiol-17-diphosphate (6), 4-iodoestradiol-17-diphosphate (7), and 2,4-diiodoestradiol-17-diphosphate (8). A solution of 17β-estradiol (35 mg, 0.13 mmol) in 20 ml of chloroform-acetonitrile was added to a stirred slurry containing phosphorus pentoxide (2.5 g) in 85% aq. phosphoric acid (3.5 ml). The resulting mixture was stirred 36 h at ambient temperature in a tightly closed flask. To this material was added an ice-cold aqueous solution of sodium hydroxide until pH 11 was reached. The resultant solution was acidified with diluted HCl to pH 5.5, and saturated with sodium chloride. After repeated extraction with 1:1 acetonitrile-ether (6x50 ml) combined organic layers were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The solid residue was examined by TLC. The double dimensional technique was utilized in the analytical manner: a plate was developed firstly in chloroform-acetone (9:1) and afterwards in 2-propanol-etanol (1:1). A spot corresponding to R_F 0.53 in the latter development was localized as well as a spot from some unreacted

starting material (Table 1). The substance having R_F 0.53 in the alcoholic solvent was extracted with ethanol from a preparative TLC plate to afford 43 mg (77 %) of 5, mp 69-70°. Mass spectral data m/e (rel. intensities): 430 (M^+, 3 %), 412 (5 %), 369 (3 %), 368 (2 %), 356 (7 %), 355 (2 %), 314 (25 %), 272 (4 %), 271 (4 %), 270 (4 %), 255 (10 %), 228 (5 %), 227 (5 %), 200 (2 %), 199 (3 %), 43 (100 %). Anal. ($C_{18}H_{24}O_8P_2$) C 50.98 %; H 5.90 % (calc. C 50.24 %; H 5.62 %). The methanolic ferric chloride reagent and pyridine gave the positive test thus confirming the retention of a phenol group in the phosphorylated product.

Following the procedure above described for preparing 5, compound 2 gave 6 (51 %), compound 3 yielded 7 (62 %), while 4 gave 8 (42 %). TLC of 6, 7 and 8 was performed following the above procedure for a double dimensional technique. Their R_F values were found to be 0.64, 0.69 and 0.72, respectively. All isolated products (6-8) gave the positive test with methanolic ferric chloride reagent.

Preparation of 4-^{125}I-estradiol-17-diphosphate (9) and 2-^{125}I,-estradiol-17-diphosphate (10). From the ethanol stock solution containing 3.4 mg of 4-iodoestradiol-17-phosphate (6) per ml. an aliquot of 10 µl (34×10^{-3} mg, 7.9×10^{-1} mmol of 6) was removed and placed in the reaction vessel. To this solution 0.25 ml of acetonitrile and 0.25 ml of ethanol was added as well as 10 uCi of $Na^{125}I$. A quantity corresponding to 10^{-2} mM of CuCl was then added and the solution was continuously stirred. At several intervals of time (2, 14, 46, 72, 96, 120, 144 h) aliquots of 10 µl were taken from the reaction mixture and were applied on a TLC plate. After a double dimensional development of a chromatogram (Table 1)silica gel corresponding to R_F 0.64 ± 0.05 was scratched from the plate. This silica was transferred to a counting vial while the rest of silica gel from the plate was placed in another vial. The activity of these samples was measured giving the rough radioiodine exchange yield. It was

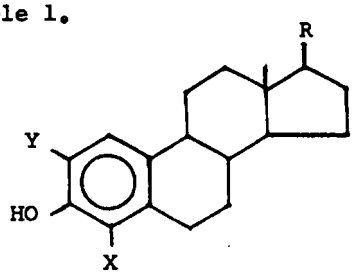
found that the maximum in the activity of the product is reached after 72 hours of the reaction course (Table 2). Thereafter, the content from the reaction vessel was applied on a preparative plate and silica corresponding to R_F 0.64±0.05 was scratched. The organic material was repeatedly extracted with EtOH. The organic solution was concentrated to 1 ml volume which was passed through the anion exchange column. Distilled water (3 ml) was used as an eluent. One tenth of the total activity remained on a column. The eluent was saturated with NaCl and was extracted with 1:1 acetonitrile-ether (6x20 ml). The combined extracts were dried (Na_2SO_4) and the solvent was evaporated *in vacuo*. Automatic gamma counter was used for radioactivity measurement of the product (Table 2). The aliquot of the isolated substance was applied on a TLC plate. R_F values of the product on radiochromatogram was identical to this for unlabeled material **6** (R_F 0.64) allowing structural assignments of the radioiodinated compound. In order to confirm the identity and purity of **9**, this substance was mixed with the unlabeled analog as carrier and the resulting mixture was analyzed on a TLC plate. Only single spot with R_F 0.64 was observed, and the radioactivity peak appeared at the same point.

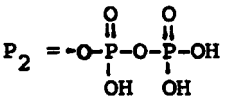
The described procedure was repeated for the compound **10** (using unlabeled diphosphate **7** as the reference). Results of these experiments (R_F values for **7** and **10** were 0.68-0.69) and yields of radioiodine exchange processes (Table 2) were identical to those above described.

RESULTS

Iodination of estradiol (**1**) leads to the formation of 4-iodoestradiol (**2**), 2-iodoestradiol (**3**), and 2,4-diiodoestradiol (**4**). It has been found recently that high-performance liquid chromatography is a suitable technique for the isolation of iodinated estradiols[15]. This work demonstrates the possibilities for isolation of these de-

rivatives using preparative TLC. Firstly, the remaining starting material was separated from the product mixture using methylene chloride. Subsequent isolation of compounds 2-4 was achieved on a TLC plate utilizing chloroform-acetone (9:1) in a single or double development technique. The latter manner seems to be preferable to a single development method. R_F values of compounds 1-4 are listed in Table 1.





	1	2	3	4	5	6	7	8	9	10
X	H	I	H	I	H	I	H	I	^{125}I	H
Y	H	H	I	I	H	H	I	I	H	^{125}I
R	OH	OH	OH	OH	P_2	P_2	P_2	P_2	P_2	P_2

In the present paper we describe also the conversion of estradiol and previously purified iodinated estradiols (2-4) to 17-diphosphates (5-8) in the reaction of these substrates with anhydrous phosphoric acid. According to published literature[22] the procedure employed in this work leads selectively to phosphoriylation of an alcoholic OH function but does not affect a phenol group. The resulting reaction mixtures obtained from these reactions were analyzed by double dimensional TLC (Table 1). Some unreacted starting material was observed as well as the major reaction product. From TLC data it appears that the latter substance is of much greater polarity in regard with the parent compound. Isolation of compounds 5-8 was carried out using preparative TLC as described in Experimental. The phenolic character of compounds 5-8 was confirmed by a reliable methanolic ferric chloride test. The grey color produced was indistinguishable from a change in the color when estradiol was tested. Molecular formula of 5 came from both mass spectral data and

elemental analysis thus making the structural assignment possible.

Table 1. TLC R_F VALUES OF ESTRADIOL, IODINATED ESTRADIOLS, AND IODINATED ESTRADIOL-17-DIPHOSPHATES

Solvent systems: (A) methylene chloride; (B) chloroform-acetone (9:1); (C) chloroform-acetone (9:1)[a] and 2-propanol-ethanol (1:1)[b]; (D) chloroform-acetone (9:1)[a] and acetone[c].

Compound	Solvent system						
	A	B	B[d]	C[a]	C[b]	D[a]	D[c]
1	0.10	0.42	0.60	0.41	0.93	0.42	0.92
2	0.20	0.48	0.65	0.46	0.95	0.47	0.94
3	0.24	0.54	0.73	0.56	0.96	0.53	0.95
4	0.31	0.63	0.81	0.62	0.97	0.63	0.96
5				0.00	0.53	0.00	0.51
6				0.00	0.61	0.00	0.60
7				0.00	0.64	0.00	0.63
8				0.00	0.68	0.00	0.66

[a] the first solvent used in a double dimensional manner; [b,c] the second solvent used in a double dimensional TLC; [d] double development technique.

When hydrolyzed in an acidic medium compounds 5-8 gave (according to TLC analysis) 1, 2, 3 and 4 respectively. Therefore, structural features of compounds 6-8 could be concluded from the results of these studies.

A cuprous ion catalyzed exchange reaction of compounds 6 and 7 and $Na^{125}I$ produced satisfactory yield of radioiodinated estradiol diphosphates (9 and 10). The best conditions were achieved using 1:1 acetonitrile:ethanol as a solvent while utilization of chloroform--2-propanol (1:1) failed to produce a significant labeling yield (Table 2). Radioiodinated products were isolated from the reaction

mixture by a double dimensional TLC technique using 9:1 chloroform-acetone and pure acetone as solvents,respectively. These systems leave only 4-5% of the background radioactivity in the area of the spots corresponding to 9 and 10 (Table 1),as found in control experiments. The remainder of inorganic radioactivity was removed in the step involving ion-exchange chromatography utilizing small columns (1 x 5 cm) and an anion-exchange resin. R_F values corresponding to radiolabeled compounds 9 and 10 were calculated from the developed radiochromatograms. Structural assignments of radioiodinated products were done by a comparison of these data (R_F values 0.61 and 0.64,respectively) with those obtained on unlabeled analogs. The relative quantities of labeled products were determined from the digital output of each area that matched a known R_F. The course of the

Table 2. RELATIVE RADIOACTIVITY OF IODINATED ESTRADIOL-17-DIPHOSPHATES IN THE EXCHANGE REACTION WITH $Na^{125}I$ IN THE PRESENCE OF CuCl

Reaction time (hours)	Compound 9[a,b]	Compound 10[a,b]	Compound 10[c]
	relative activity (%)		
2	3.8	4.6	2.4
14	6.2	8.2	4.2
46	24.0	22.0	7.4
72	39.0	36.1	8.1
96	33.5	35.8	8.0
120	18.0	17.0	
144	13.0	12.0	

[a] 1:1 acetonitrile-ethanol as a solvent; [b] specific activities of both 9 and 10 were 125 μCi/mg starting with 10 μCi of $Na^{125}I$,10^{-2} mM of CuCl,and $8x10^{-1}$ mM of either 6 or 7; [c] 1:1 chloroform-2-propanol as a solvent.

reaction was followed by measuring the relative distribution ratio of activity between areas on plates where products were located and

the rest of radioactivity corresponding either to radioiodide or to radioiodinated estradiols. The radioactivity equilibrium between the labeled substrate and the radioiodide was reached after 3 days and was determined as 35-39 % for both 9 and 10 in three independent experiments (Table 2).

The stability study of radioiodinated products was no carried out. Inspecting the data in Table 2 it seems that both compounds are of a rather low stability as indicated before for monophosphate an - logs[14]. Therefore, storage in diluted solutions and at dry-ice temperature is highly recommended for the radioiodinated steroids described here.

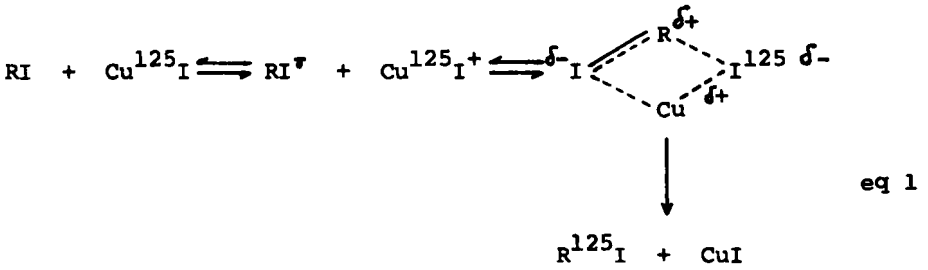
DISCUSSION

The conversion of estradiol to its mono- and diiodinated derivatives by electrophilic substitution of positive iodine in the activated aromatic ring is the well established process. More attention should be focused on the phosphorylation of compounds 5-8.

More than twenty five years ago the method for the preparation of pyridoxal phosphate utilizing anhydrous H_3PO_4 was described[23]. When compared with the preparation of the same substrate using phosphorous oxychloride, the former procedure proved to be superior respecting the yield of the product obtained. However, elemental analysis suggests that the isolated material may be contaminated with the diphosphate as a byproduct[23]. In the synthesis of 5 it was found that the use of concentrated solutions of estradiol or the application of ethanol as a solvent leads to the formation of products of much greater polarity. This material gave positive ferric chloride test but remained on the start when developed in alcoholic solvents (or in acetone) on TLC. During a storage at room temperature the unidentified product decomposes within few days giving a number of spots on a TLC plate (R_F values from 0 to 0.55 in 2-propanol-ethanol

1:1). These data indicate that the change in the reaction conditions drasticaly influences the product composition favoring probably the formation of polyphosphates. Similar results were obtained by prolonging the reaction time.

Isotopic exchange reaction of monoiodinated estradiol-17-diphosphates and $Na^{125}I$ was observed only using CuCl as a catalyst while no radioactivity associated with 6 and 7 was found in the presence of zinc chloride or cupric chloride. Cuprous ion is a reductive agent in the couple $Cu^{+} \rightleftharpoons Cu^{2+} + e^{-}$ at the standard potential $E^{o} = 0.16$ V. The actual potential of this couple depends on the equilibrium point, i.e. on the relative ratio between the reduced and oxidized form[24]. The carbon-iodine bond as one of the electrochemically most reactive bonds undergoes a reduction with relative ease. Bearing in mind the postulate that raduction takes place in one electron processes[25], the potential determining step involves the transfer of the first electron to the LUMO σ^{*} frontier orbital of the carbon-halogen bond[26]. The first order rate measured in the radioiodine exchange of monoiodinated diethylstilbestrols using alcoholic solvents[17,27] is in accordance with the first order kinetics in electron transfer processes. From these data we assume that the electron transfer step is also rate determining. The best rationalization of the reported results is, in our opinion, the formation of a four center reaction transition state[27] leading to



eq 1

the iodide isotope exchange (eq 1). The polarity of solvents used stabilizes probably the quadrupole state (eq 1)[28], thus favoring the proposed mechanism over an usual $S_{NR}1$ route[29] (eq 2).

$$RI + Cu^{125}I \rightleftharpoons RI^{\bullet-} + Cu^{125}I^{+} \rightleftharpoons R\bullet + CuI^{125}I \rightleftharpoons$$

$$\rightleftharpoons R^{125}I^{\bullet-} + CuI^{+} \xrightarrow{+RI} R^{125}I + RI^{\bullet-} + CuI^{+} \qquad \text{eq 2}$$

The above sequence (eq 2) does not parallel the observed monomolecularity and the first order rate of exchange reactions[27] since the $S_{NR}1$ path favors propagation over termination steps. The absence of radical coupling products in both the reaction studied and in the exchange labeling of iodinated stilbestrols[17] further supports equation 1 as the plausible mechanistic interpretation.

From the synthetic point of view it seems that monoiodoestradiol diphosphates can be easily radioiodinated with $^{125}I^{-}$ using the described method. However, the synthetic scopes of this exchange reaction for labeling the substrate with short-lived radionuclides is quite limiting. The direct labeling technique should be more appropriate in both achieving high specific activities and a suitable reaction time. Preliminary studies on direct labeling of numerous steroids using appropriate redox couples are now in progress in our laboratory. The results of this work will be published elsewhere.

REFERENCES

1. Tarle M., Cvrtila D., Padovan R., and Spaventi Š., - Preliminary report on radioiodination of monoiodoestradiol diphosphates and their in vitro activity, paper presented at 15th International Annual Meeting, Society of Nuclear Medicine, Groningen, Sept. 13-16, 1977.
2. Institute of Tumors and Allied Diseases, Zagreb, Yugoslavia.

3. King R.J.B. and Mainwaring W.I.P. - Steroid-cell Interactions, Butterworths, London, 1974.

4. Skinner R.W., Pozderac R.V., Counsell R.E., and Weinhold P.A. - Steroids, 25, 189 (1975).

5. Wilson E.M. and French F.S. - J.Biol.Chem., 251, 5620 (1976).

6. Rennie P. and Bruchovsky N. - J.Biol.Chem. 248, 3288 (1973).

7. Unhjem O. and Tveter K.J. - Acta Endocrinol., 60, 571 (1969).

8. Fang S., Anderson K.M., and Liao S. - J.Biol.Chem., 244, 6584 (1969).

9. (a) Tubis M., Endrow J.S., and Blahd W.H. - Nucl.Med., 6, 1 (1967).
(b) Tubis M., Endrow J.S., and Blahd W.H. - Nucl.Med., 6, 184 (1967).

10. Szendroi Z., Koczar L., Karika Z., and Eckhardt S. - Lancet, 1, 1253 (1973).

11. Pennisi F. and Rosa U. - J.Biol.Nucl.Med., 13, 64 (1969).

12. (a) Ghanadian R., Waters S.L., Thakur M.L., and Chisholm G.D. - Int.J.Appl.Rad.Isot., 26, 343 (1975).
(b) Ghanadian R., Waters S.L., Williams G., and Chisholm G.D. - Eur.J.Nucl.Med., 1, 159 (1976).

13. Waters S.L. - personal communication.

14. Shida K., Shimazaki J., Kurihara H., Ito Y., Yamanaka H., and Furuya N. - Int.J.Nucl.Med.Biol., 3, 86 (1976).

15. Maysinger D., Marcus C.S., Wolf W., Tarle M., and Casanova J. - J.Chromat., 130, 129 (1977).

16. Maysinger D., Wolf W., Casanova J., and Tarle M. - Croat.Chem. Acta, 49, 123 (1977).

17. Maysinger D., Tarle M., Marcus C.S., Casanova J., and Wolf W. - Abstract of Papers, 1st Chemical Congress of the North American Continent, Mexico City, Nov. 30-Dec. 5, 1975.

18. Maysinger D., Tarle M., Wolf W. and Markland F. - Abstract of Papers, C-1 272, Meeting of Chemists of Croatia, Zagreb,

Febr. 16 - 18,1977.

19. (a) Maysinger D.,Wolf W.,Casanova J.,and Tarle M., - Croat. Chem.Acta,49, 549 (1977).
(b) Ferguson L.N., Tarle M., Wolf W., Lien E.J., and Maysinger D., - Bull.Soc.Chim.Beograd, 42, 147 (1977).

20. Although SBP does not play a role in steroid action at the cellular level it facilitates the transport of a hormone to a sex gland[21].

21. Mercier-Bodard C., Alfsen A.,and Baulien E.E., - Acta Endocrin., Kbh, Suppl., 147, 204 (1970).

22. Komai T., Eckelman W.C., Johnsonbaugh R.E., Mazaitis A., Kubota H., and Reba R.C., - J.Nucl.Med., 18, 360 (1977).

23. Wilson A.N. and Harris S.A., - J.Am.Chem.Soc., 73, 4693 (1951).

24. The actual potential can be predicted by the Nernst equation: $E = E^{o} - RT/nF(\ln(c_{red}/c_{ox}))$.

25. Rifi M.R., - "Reduction of Organic Halides",in "Organic Electrochemistry", M.M. Baizer, ed., Marcel Dekker,Inc., New York, 1973, p 278.

26. Fukui K., Morokuma K., Kato H., and Youzewa T., - Bull.Chem. Soc.Japan, 36, 217 (1963).

27. Maysinger D., Wolf W., Marcus C.S., Casanova J., and Tarle M., - Int.J.Appl.Rad.Isot., paper submitted for publication.

28. The strong dependance of the radioactivity yield on the polarity of a solvent used in the radioiodination of the 11-I_1--17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (RU 5020) was also observed in our laboratory.

29. Kim J.K. and Bunnett J.F., - J.Am.Chem.Soc., 92, 7463 (1970).