Radioiodinated Ligands for the Estrogen Receptor: Effect of 3-*o*-Methylation on Tissue Distribution

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The 3-*o*-methyl ethers of 17α-[^{125}I]iodovinylestradiol and 17α-[^{125}I]iodovinyl-11β-methoxyestradiol were prepared in 95% and 89% isolated yields, respectively, at the no-carrier-added level from the corresponding (tri-*n*-butystannyl)-vinyl intermediates. The new radioligands were evaluated in immature female rats to determine their uptake in, and selectivity for, estrogen-receptor-containing tissues. At 0.5–6 hr after administration, both agents showed preferential uptake and retention by the target tissue. The values for the 11β-methoxy derivative, however, were significantly better than those of the 11-unsubstituted compound. Compared with the parent 3-hydroxy radioligands, the [^{125}I]VE_2-3-*o*-Me had lower uptake and target-to-blood ratios at all time periods, but by 6 hr the [^{125}I]VME_2-3-*o*-Me compound showed as high an uptake in the uterus and higher uterus-to-blood ratios. This may be related to metabolic cleavage of the 3-*o*-methyl group generating the parent compound, which is then sequestered by the target tissue. The results suggest that iodine-123-labeled VME_2-3-*o*-Me would be a good candidate for in vivo gamma imaging of estrogen-containing tissues.

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The presence of significant levels of specific hormone receptors in human breast cancer has important ramifications with regard to both the choice of appropriate therapy and the long-term prognosis of the patient (*1–5*). It has been a major objective of research efforts to detect noninvasively the neoplastic lesions, both primary and metastatic, and to determine the presence and approximate levels of the steroid receptors. One approach has involved the preparation of gamma- or positron-emitting tracers that would localize in the tissue by selectively binding to the estrogen receptors. In earlier studies we have prepared two such potential radioligands, 17α-[^{125}I]iodovinylestradiol ([I-125] VE_2) (*6*), and 17α-[^{125}I]iodovinyl-11β-methoxyestradiol ([I-125] VME_2) (*7*), and have assessed their biodistribution in immature female rats. Other investigators have also reported the preparation and evaluation of estrogenic radioligands—labeled (or potentially labeled) with I-125, I-123 (*8–13*), Br-77, Br-82 (*14–18*), or F-18 (*19–22*)—that show selectivity for tissues containing estrogen receptors.

In this study we report the effects of 3-*o*-methylation upon the distribution and selectivity of the radiolabeled estrogens. Although it has been well established that the formation of the methyl ether drastically reduces binding to the receptor in vitro, the 3-*o*-methyl ethers display substantial estrogenic activity in vivo, presumably because *o*-demethylation in the liver is rapid and provides the parent compound. The rationale for this study was therefore to evaluate the 3-*o*-methyl ethers as "prodrugs" for the radioligands that we have previously examined. The gradual conversion of the "inactive" radiochemical to the form that is actively accumulated in the target tissue may result in target-to-nontarget tissue ratios higher than with the parent compound. As the results indicate, this approach is partially successful because at least in the case of [I-125] VME_2, *o*-methylation produces an agent that at the later time points

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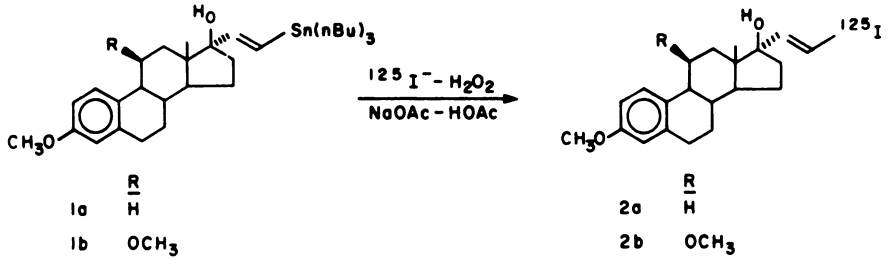

FIG. 1. Synthesis of 17α-[^{125}I]iodovinylestradiol-3-methyl ether, **2a**, and 17α-[^{125}I]iodovinyl-11β-methoxyestradiol-3-methyl ether, **2b**.

has distribution characteristics comparable to those of the parent compound.

MATERIALS AND METHODS

The preparation of the (tri-*n*-butylstannyl)vinyl precursors from estrone-3-methyl ether and RU-2660* with trans-1,2-bis(tri-*n*-butylstannyl)ethylene (*22*) proceeded in 40–50% yields, and will be described in greater detail elsewhere. All solvents were high performance liquid chromatography (HPLC) grade and were used without further purification. Sodium [^{125}I]iodide (2200 Ci/mmol) was purchased. High-performance liquid chromatography was performed with a gradient unit consisting of a microprocessor, a printer-plotter, two pumps, a constant-wavelength uv detector (254 nm), a radiometric detector and an automated fraction collector. Radioactivity in the fractions was determined using either a sodium iodide well counter or a dose calibrator. The radioactivity in tissue samples was assayed in a sodium iodide well counter.

Radioiodination procedure. To 10 μl of a 0.08 *N* NaOH solution containing 5.0 mCi (2.27 nmol) Na^{125}I in a sealed conical vial were added 25 μl of the solution containing 300–400 nmol of the 17α-(tri-*n*-butylstannyl)vinylestradiol (**1a** or **1b**, Fig. 1) and 50 μl of a solution of NaOAc in glacial acetic acid. To this was then added 50 μl of an oxidant solution consisting of H_2O_2 and acetic acid. After stirring at ambient temperature in the dark for 30 min, the reaction was terminated by the addition of 25 μl of 5% $NaHSO_3$ in water (W/V). The entire contents of the reaction vessel were extracted with a syringe and injected onto a reverse-phase HPLC column. Elution of the column with an ethanol/ammonium phosphate gradient gave the desired product. The isolated radiochemical yields for **2a** and **2b** (Fig. 1) were 95% and 89%, respectively. The identity of the isolated products was confirmed by their coinjection with authentic unlabeled **2a** and **2b** onto a Whatman PXS 10/25 ODS analytical column. The products were dissolved in an ethanol/0.9% saline solution (1:9 V/V), and stored at 4°C in the dark before their in vivo evaluation. Under these conditions deiodination was less than 2% per month.

In vivo studies. Immature female Sprague-Dawley rats (21–25 days, 45–55 g) were injected by tail vein, under light ether anesthesia, with 0.1 ml of the ethanol/saline solution containing the appropriate radiopharmaceutical (10–15 μCi). Groups of rats (N = 5) were killed at 0.5–6 hr after injection, and samples of blood and tissues were removed, weighed, and assayed for activity. Tissue and blood levels of activity were calculated as percent dose x animal weight (kg) per gram of tissue or blood (% dose × kg/g). Uterus-to-blood ratios at the various points were calculated for both **2a** and **2b**.

To determine the specific binding in vivo, groups of immature female rats (21–25 days, 45–54 g, 5–6 per group) were injected with 0.2 ml of an ethanol/saline solution containing 10 μCi of the appropriate radiochemical, with or without 50 μg estradiol. The groups of rats were killed at 2 hr after injection and the samples of blood and tissue were removed and analyzed as previously described. The difference in tissue levels in the absence or presence of estradiol was taken as representing specific estrogen-receptor binding.

RESULTS

Radiosynthesis of 17α-[^{125}I]iodovinylestradiol-3-methyl ether ([^{125}I]VE$_2$-3-*o*-Me) 2a and 17α-[^{125}I]iodovinyl-11β-methoxyestradiol-3-methyl ether ([^{125}I]-VME$_2$-3-*o*-Me) 2b. The desired compounds were prepared according to the scheme outlined in Fig. 1. For both radioligands, a large excess (132–176:1) of the stannyl precursor relative to the radioiodide was used. The reaction was terminated at 30 min and the separation of product from the starting materials was achieved by HPLC. As the chromatograms in Fig. 2 indicate, the separation was complete and the excess starting material posed no problem, since it eluted long after the product. The other components of the reaction mixture also eluted at times well differentiated from those of the product. Comparison of the radiopharmaceuticals—isolated in 95% and 89% yields, respectively—by coinjection with the corresponding unlabeled iodovinylestradiol-3-methyl ethers confirmed that the iodination had proceeded at the position indicated and not elsewhere on the molecule.

Tissue uptake and selectivity in immature rats. The concentration of radioactivity in the tissues of interest,

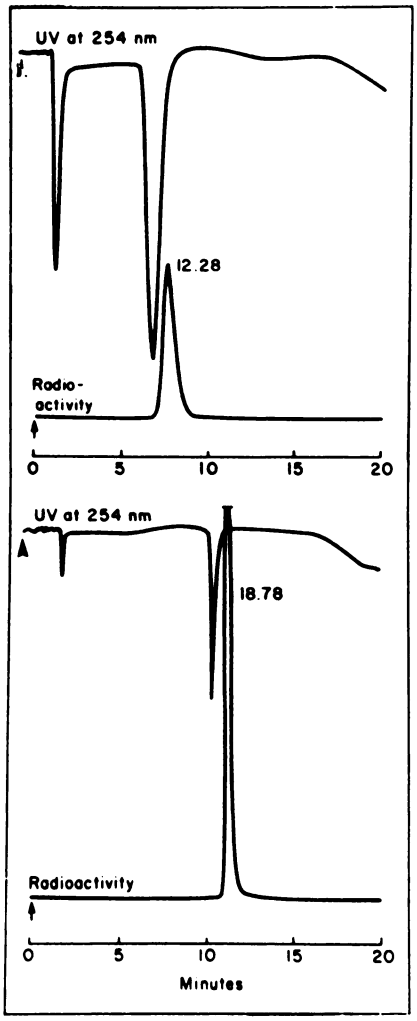

FIG. 2. HPLC analysis of **2a** (top) and **2b** (bottom) coinjected with authentic unlabeled **2a** and **2b**. Elution was performed on Whatman PXS ODS 10/25 analytical column with linear gradient consisting of 45% ethanol/55% 5 m*M* $(NH_4)H_2PO_4$ at t = 0 min, to 90% ethanol/10% 5 m*M* $(NH_4)H_2PO_4$ at t = 45 min; flow rate = 1 ml/min; uv detector at 254 nm, 0.032 absorbance units full scale (AUFS). Under these conditions stannylated percursors elute at >45 min.

and the uterus-to-nontarget tissue ratios are shown in Tables 1 and 2. The values previously obtained for the corresponding 3-hydroxy compounds are included for comparison (*6*,*7*). The level of activity in the uterus increased from 0.179 to 0.286 %dose × kg/g for **2b**, whereas levels of activity declined in virtually all other tissues. Although the overall tissue distribution patterns for the methyl ethers and the parent estrogens were similar, there were some important differences, particularly in uterine uptake. The peak uterine level of [I-125]VE_2 was greater than that of its methyl ether by almost a factor of two (0.465 compared with 0.286); but the methyl ether had a relatively greater percentage of peak activity at 4 hr than did the parent compound (77% compared with 43%). For the 11β-methoxy-substituted agent the differences in the kinetics of uterine uptake and retention were even more pronounced. Uptake of the parent radioligand was maximal at 1 hr after injection, and was over three times that of the methyl ether. However, the uterine levels of activity following the administration of the methyl ether continued to rise, reaching their highest measured values at 6 hr, and were comparable to or slightly greater than those of the parent compound.

The tissue-to-blood ratios shown in Table 2 reflect these observations. The values for[I-125]VE_2-3-*o*-Me rose and declined more slowly than those of [I-125]VE_2, but at no time did the uterus-to-blood ratios of the ether exceed those of the parent. For the 11β-methoxyestradiols, both compounds showed improvement in their uterus-to-blood ratios over the 1- to 6-hr period. The primary difference, however, was that the methyl ether increased by 24-69:1, about a threefold increase, whereas the improvement for [I-125]VME_2 was only 50%, from 43-61:1. The other tissue-to-blood ratios for the methyl ethers, compared with the 3-hydroxy parent compounds, were not essentially different.

The effect of coadministered estradiol upon the tissue distribution of **2a** and **2b** is shown in Table 2. As the data indicate, the only tissue in which the localization is significantly depressed is the uterus, where the levels are decreased 50% and 73%, respectively. The amount of nonspecific binding in the uterus is approximately equal for the two compounds, but the concentration of activity in the absence of estradiol for the 11-β-methoxy compound (**2b**) was substantially greater than that for **2a** (0.59 compared with 0.32 %dose × kg/g). Uptake in the other nontarget tissues is largely unaffected, or marginally enhanced, by the coinjection of receptor-saturating doses of estradiol.

DISCUSSION

In this study we have described the synthesis of two radiolabeled estrogens at the no-carrier-added level by means of the radioiododestannylation method. This procedure results in the highly efficient and rapid preparation and purification of the desired compounds. The target compounds in this case incorporate, at the 3-position of the estrogen, a methyl ether that is metabolically labile and will generate the parent estradiol in the intact organism.

The uptake and selectivity of the two methyl ethers were compared with those previously observed and reported for the parent estradiol derivatives. Because structurally similar 17α-ethynylestradiol 3-methyl ethers show significant in vivo uterotropic activity, it was expected that both agents would be accumulated and selectively retained by the uterus in the immature female rat. The presence of the *o*-methyl group has two effects: a biological one that reduces receptor affinity and requires cleavage to produce a high-affinity metabolite, and a physiochemical one that increases lipophilicity and

TABLE 1. TISSUE DISTRIBUTION OF I-125 ACTIVITY AFTER INTRAVENOUS ADMINISTRATION OF RADIOLABELED ESTROGENS IN IMMATURE FEMALE RATS

Agent	Time (hr)	Uterus	Liver	Lungs	Muscle	Blood
[I-125] VE_2	0.5	0.212*	0.324	0.081	0.063	0.046
		±0.020	±0.014	±0.004	±0.004	±0.005
	1	0.434	0.144	0.055	0.044	0.027
		±0.099	±0.024	±0.003	±0.010	±0.002
	2	0.465	0.105	0.046	0.043	0.023
		±0.085	±0.009	±0.009	±0.005	±0.001
	4	0.200	0.074	0.025	0.024	0.026
		±0.020	±0.008	±0.002	±0.003	±0.002
[I-125] VE_2-3-*o*-Me **2a**	0.5	0.179	0.467	0.246	0.136	0.052
		±0.017	±0.036	±0.019	±0.010	±0.004
	1	0.198	0.262	0.158	0.090	0.031
		±0.027	±.019	±0.016	±0.009	±0.004
	2	0.286	0.162	0.092	0.049	0.022
		±0.098	±0.016	±0.008	±0.005	±0.003
	4	0.220	0.131	0.048	0.028	0.035
		±0.090	±0.015	±0.005	±0.004	±0.008
[I-125] VME_2	1	0.821	0.146	0.079	0.063	0.019
		±0.138	±0.014	±0.009	±0.007	±0.002
	2	0.751	0.117	0.050	0.074	0.013
		±0.072	±0.008	±0.004	±0.012	±0.001
	6	0.704	0.085	0.027	0.027	0.012
		±0.106	±0.008	±0.003	±0.004	±0.001
[I-125] VME-$_2$-3-*o*-Me **2b**	1	0.264	0.117	0.071	0.045	0.011
		±0.022	±0.017	±0.006	±0.005	±0.001
	2	0.425	0.103	0.061	0.040	0.012
		±0.003	±0.009	±0.005	±0.004	±0.001
	6	0.760	0.105	0.034	0.049	0.011
		±0.049	±0.011	±0.002	±0.005	±0.001

* Mean % dose × kg/g ± s.e.m., for n ≥ 5 animals.

results in greater initial distribution to the nontarget tissues. It is not surprising, therefore, that the observed uptake in the uterus at the early time periods, 0.2–5 hr, is lower than that of the parent 3-hydroxy compounds. That the localization of the radioactivity in the uterus proceeded by way of a specific receptor-mediated process is shown by the study using a large excess of the unlabeled estradiol. If the preferential uptake in target tissue resulted from a nonspecific physiochemical interaction involving the intact 3-*o*-methyl group, the presence of the high levels of estradiol should have little or no effect. On the contrary, because a high percentage of the uptake in the uterus is displaced by the estradiol, specific estrogen-receptor binding, which requires the free 3-hydroxy group, must be present. It is probable, therefore, that it is the parent radiolabeled estrogen and not the 3-*o*-methyl compound that is accumulated in the target tissue. In that sense the agents **2a** and **2b** are functioning as radioactive "prodrugs."

The effect produced by the presence of the 11β-methoxy group on the pharmacokinetics of the methyl ethers is greater than anticipated, but can be justified using the explanation of Raynaud et al. (*24*) for the H-3-labeled 17α-ethynyl analogs. Following i.v. administration, both agents were rapidly distributed. Some of the unmetabolized agent enters the uterus, but because it has a low affinity for the receptor, it is not retained at this time. Passage through the liver results in *o*-demethylation by metabolic enzymes, forming the active radiolabeled estrogen. At this point the fate of the

TABLE 2. TISSUE-TO-BLOOD RATIOS FOLLOWING INTRAVENOUS ADMINISTRATION OF RADIOLABELED ESTROGENS IN IMMATURE FEMALE RATS

Agent	Time (hr)	Uterus	Liver	Lung	Muscle
[I-125] VE_2	0.5	4.6	7.0	1.8	1.5
	1	16.1	5.3	2.0	1.6
	2	20.2	4.6	2.0	1.9
	4	7.7	2.8	1.0	1.0
[I-125] VE_2 3-*o*-Me	0.5	3.5	9.0	4.7	2.6
	1	6.4	8.5	5.1	2.9
	2	13.0	7.4	4.2	2.2
	4	6.3	3.7	1.4	0.8
[I-125] VME_2	1	43.0	7.7	4.2	3.3
	2	56.1	8.8	3.8	3.6
	6	60.7	7.1	2.3	2.3
[I-125] VME_2 3-*o*-Me	1	24.0	10.6	6.5	4.1
	2	35.4	8.6	8.6	3.3
	6	69.1	9.5	3.1	4.5

two radiochemicals would differ significantly. As has been described earlier by Raynaud, the 11β-methoxy group suppresses both the binding to proteins and the proportion of the less-active metabolites in the plasma relative to the 11-unsubstituted estrogens. Therefore, there is a much greater concentration of radioligand available for uptake and binding to the uterus. Because the overall clearance of the 11β-methoxyestrogen from the plasma is slower, the accumulation process in the uterus will continue for a longer period than for the 11-unsubstituted estrogen. As the data obtained in this study indicate, the activity in the uterus following the administration of **2b** continues to increase over the 6-hr time frame until it is not only much greater than that of the 11-unsubstituted methyl ether, but is also equal to or greater than that observed for the parent compound [I-125]VME_2. This increased uptake over time proceeded with very low concentrations of circulating ac-

TABLE 3. DISTRIBUTION OF I-125 ACTIVITY IN IMMATURE FEMALE RATS 2 hr AFTER INTRAVENOUS ADMINISTRATION OF I-125-LABELED VE_2-3-*o*-Me (2a) AND VME_2-3-*o*-Me (2b) IN ABSENCE OR PRESENCE OF ESTRADIOL

Tissue	[I-125] VE_2-3-*o*-Me 2a			[I-125] VME_2-3-*o*-Me 2b		
	$-E_2$	$+50\ \mu g E_2$	% Change	$-E_2$	$+50\ \mu g E_2$	% Change
Uterus	0.32*	0.16	−50	0.59	0.16	−73
	±0.11	±0.06		±0.02	±0.02	
Ovaries	0.25	0.27	+8	0.31	0.24	−23
	±0.06	±0.04		±0.03	±0.03	
Liver	0.17	0.25	+47	0.088	0.090	+2
	±0.03	±0.03		±0.009	±0.009	
Lung	0.090	0.123	+37	0.074	0.079	+7
	±0.020	±0.012		±0.009	±0.005	
Muscle	0.053	0.057	+8	0.068	0.073	+7
	±0.003	±0.004		±0.011	±0.005	
Blood	0.023	0.031	+35	0.017	0.020	+18
	±0.003	±0.002		±0.002	±0.001	

* Mean % dose × kg/g ± s.e.m., for n ≥5 animals.

tivity (0.11 %dose × kg/g), implying that the accumulation of the agent in the uterus occurs against a substantial concentration gradient. In this regard we note that the uterus-to-blood ratio observed is greater than that obtained by Eckelman et al. (*25*) for potent H-3-labeled estrogenic ligands (69:1 compared with 26-57:1).

In summary, we have described the preparation of two radioiodinated estradiol-3-*o*-methyl ether derivatives in high radiochemical yields at the no-carrier-added level, and their purification by HPLC. The evaluation of these agents in immature female rats as potential imaging agents for tissues containing estrogen receptors revealed some interesting substituent effects both for the 3-*o*-methyl group and the 11β-methoxy moiety. The radioiodinated IVME$_2$-3-*o*-Me shows substantial potential as an imaging agent and will continue to be investigated along with the 3-hydroxy parent compound.

FOOTNOTE

* Roussel-Uclaf.

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