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17α-Z-[^{125}I]IODOVINYLOESTRADIOL AND ITS 3-ACETATE: CHEMICAL SYNTHESIS AND *IN VIVO* DISTRIBUTION STUDIES IN THE RAT

COMPARISON OF TISSUE ACCUMULATION AND METABOLIC STABILITY WITH 17α-E-[^{125}I]IODOVINYL AND 16α-[^{125}I]IODO OESTRADIOLS

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Abstract—Certain oestrogens on substitution with halogens, have been shown to retain receptor binding affinity and accumulate in target tissues and therefore could, when labelled with γ-emitting halogen isotope, act as radiopharmaceuticals for the imaging of receptor positive breast tumours. In order to evaluate putative radiopharmaceuticals, 17α-Z-iodovinyloestradiol ((17α,20Z)-21-iodo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol) and its 3-acetate have been chemically synthesized and labelled with ^{125}I. Tissue distribution studies in female rats have been compared to those obtained using 17α-E-[^{125}I]iodovinyloestradiol ((17α,20E)-21-[^{125}I]iodo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol) and its 3-acetate and to 16α-[^{125}I]iodo oestradiol (1,3,5(10)-estratrien-16α-[^{125}I]iodo-3,17β-diol). All compounds showed a similar tissue distribution with the highest concentrations (cpm/g tissue) in the thyroid > liver > uterus > kidney > lung > blood > heart, spleen. The concentration in the uterus was highest after injection of 17α-Z-iodovinyloestradiols or 16α-iodo oestradiol. Target (uterus) to background (blood) tissue ratios were highest after injection of 17α-Z-iodovinyloestradiol-3-acetate and 16α-iodo oestradiol (6:1). Deiodination *in vivo* took place to a small degree, amounting to 1.6%, 0.9% and 0.35% of the injected dose after 4 hr with the 17α-Z, 17α-E and 16α compounds, respectively. For reasons of chemical expediency and consideration of the biological results 17α-Z-iodovinyloestradiol-3-acetate is the compound of choice for the detection of oestrogen receptor positive tissues.

In recent years a number of γ-emitting halogenated derivatives of oestradiol have been synthesized at very high specific activities. These include 1,3,5(10)-estratrien-16α-iodo-3,17β-diol[16α-iodo-oestradiol] and (17α,20E)-21-iodo-19-norpregna-1,3,5(10),20-tetraene - 3,17β - diol [17α - E-iodovinyloestradiol] with high affinity for the oestrogen receptor [1–3]. The primary motivation has been the development of oestrogen receptor (ER) directed imaging agents for the detection of oestrogen receptor positive breast tumours [4, 5]. Although some excellent examples of images have been obtained with these compounds [6, 7], other studies have given more disappointing results [8, 9].

Two further developments have occurred which may lead to improved radiopharmaceuticals. The first is the demonstration that an additional modification to the steroid nucleus, the introduction of a methoxy group at the 11β position, greatly improves target tissue uptake and retention [10–12] of 16α-iodo oestradiol and 17α-E-iodovinyloestradiol. The second is the synthesis of the (17α,20Z)-21-iodo-19-norpregna-1,3,5(10),20-tetraene - 3,17β-diol [17α-Z-iodovinyloestradiol] isomer [13] which is more avidly bound by oestrogen receptor and accumulates in oestrogen sensitive tissues approximately five times better than the 17α-E isomer.

Both 17α-Z- and 17α-E-iodovinyloestradiol were chemically synthesized via hydrolysis of their corresponding 3-acetates. We have previously shown that the acetate is rapidly removed *in vivo* [14]. Therefore, this chemical step could be omitted, an advantage in the preparation of reagents labelled with an isotope of short half life such as ^{123}I. The 17α-Z-iodide has been shown to be less stable *in vivo* than the corresponding 17α-E isomer but there are no published data on the stability of the 16α-iodide. In order to evaluate further, compounds for the imaging of ER positive tissues we have prepared ^{125}I-labelled 17α-Z-iodovinyl and 17α-E-iodovinyloestradiol and their 3-acetates and compared their tissue distribution, including the thyroid, in young mature female rats with that of 16α-[^{125}I]iodo oestradiol.

MATERIALS AND METHODS

All materials were used as supplied except diethyl

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‖ Abbreviations: 17α-Z-iodovinyloestradiol, (17α,20Z)-21-iodo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol; 17α-E-iodovinyloestradiol, (17α,20E)-21-iodo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol; 16α-iodo oestradiol, 1,3,5(10)-estratrien-16α-iodo-3,17β-diol; 16α-iodo oestrone, 1,3,5(10)-estratrien-16α-iodo-3-hydroxy-17-one; ER, oestrogen receptor.

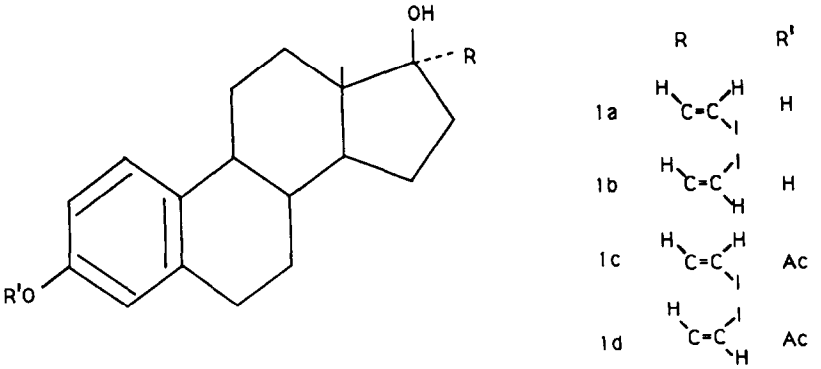

Fig. 1. Chemical structures of (1a) 17α-*Z*-iodovinyloestradiol, (1b) 17α-*E*-iodovinyloestradiol, (1c) 17α-*Z*-iodovinyloestradiol-3-acetate and (1d) 17α-*E*-iodovinyloestradiol-3-acetate.

ether which was dried over sodium. Column chromatography was performed over silica gel using ethyl acetate/heptane (1:1) as eluant. NMR was performed at 200 or 400 MHz in $CDCl_3$ on a Varian VXR 200 or VXR 400 as indicated. Mass spectrometry was performed on a VG 7070H with a Finnigan Super INCOS data system. All reagents were of Analar grade. Na[^{125}I]I and 16α-[^{125}I]-iodo oestradiol were purchased from Amersham International.

Preparation of 17α-E-*tributylstannylvinyloestradiol.* Ethynyl oestradiol (215 mg, 0.72 mmol), tributyl tin hydride (215 mg, 0.72 mmol) and dibenzoyl peroxide (1.7 mg, 7.5 mmol) were dissolved in diethyl ether (5 mL) and photolysed under nitrogen using a medium pressure mercury arc lamp. The solvent was removed and the mixture purified by column chromatography to give 365 mg (86%) of the desired 17α-*E*-tributylstannylvinyloestradiol. NMR (400 MHz): 0.7–2.1, m, 44H, steroid envelope and Bu_3Sn-, 2.65–2.75, m, 2H, C-6 methylene, 5.9–6.15, dd, 2H, J = 19 Hz, vinyl CH, 6.7–7.2, m, 3H, aromatic nucleus. Mass spectrum base peak at m/e = 531; M^+-Bu.

Preparation of 17α-E-*tributylstannylvinyloestradiol-3-acetate.* 17α-*E*-Tributylstannylvinyloestradiol (24 mg, 0.35 mmol) was dissolved in pyridine (0.7 mL) and acetic anhydride (0.66 mL, 0.7 mmol) added. The mixture was allowed to stand at room temperature overnight and then partitioned between ether (50 mL) and water (50 mL). The ether layer was separated and washed twice with water (50 mL), dried ($MgSO_4$) and the solvent removed. The residue was purified by column chromatography to give 168 mg (74%) of the desired 17α-*E*-tributyl stannylvinyloestradiol-3-acetate. NMR (400 MHz): 0.7–2.1, m, 44 H, steroid envelope and Bu_3Sn-, 2.2, s, 3H, CH_3-CO, 2.7–2.8, m, 2H, C-6 methylene, 5.9–6.15, dd, 2H, J = 19Hz, vinyl CH, 6.7–7.2, m, 3H, aromatic nucleus. Mass spectrum base peak at m/e = 572; M^+-Bu + H.

Preparation of 17α-Z-*tributylstannylvinyloestradiol-3-acetate.* Ethynyl oestradiol (144 mg, 0.49 mmol) and tributyl tin hydride (432 mg, 1.48 mmol) were dissolved in hexamethyl phosphoramide (1.75 mL) and heated at 70° for 72 hr. The mixture was partitioned between ethyl acetate (50 mL) and water (50 mL). The organic layer was separated, dried ($MgSO_4$) and the solvent removed. Purification by column chromatography gave 73 mg

Table 1. Concentration of radioactivity (cpm/g × 10^{-2}) in tissues of young mature female rats (100 g) after i.v. injection of (a) 17α-*Z*-[^{125}I]iodovinyloestradiol, (b) 17α-*E*-[^{125}I]iodovinyloestradiol, (c) 16α-[^{125}I]iodo oestradiol, (d) 17α-*Z*-[^{125}I]iodovinyloestradiol-3-acetate, (e) 17α-*E*-[^{125}I]iodovinyloestradiol-3-acetate and (f) 16α-[^{125}I]iodo oestradiol to 200 g rats

Time post injections (min)	(a)				(b)				(c)			
	30	60	120	240	30	60	120	240	30	60	120	240
Uterus	23	104	88	80	32	48	28	22	145	109	82	66
Blood	20	45	35	23	21	20	16	15	24	16	16	16
Thyroid	1790	1654	7455	20,647	572	1502	3938	5290	215	275	1594	3966
Liver	43	165	275	95	158	200	105	93	173	119	118	102
Kidney	22	75	40	27	68	60	38	37	74	49	37	22
Heart	13	36	18	14	46	42	24	22	72	31	18	8
Lung	22	76	42	30	52	52	36	31	77	38	25	11
Spleen	12	36	23	16	15	23	18	13	35	20	15	7

(25%) of mixed 17α-*E*/*Z*-tributylstannyloestradiols. Acetylation by the above procedure followed by preparative HPLC over silica gel using ethyl acetate/hexane (1:5) gave 20 mg (6.5%) of the desired 17α-*Z*-tributylstannylvinyloestradiol - 3 - acetate. NMR: 0.8–2.2, m, 44H, steroid envelope and Bu_3Sn-, 2.25, s, 3H, CH_3-CO, 5.82–5.86, d, 1H, vinylic CH, J = 13Hz, 6.77–7.26, m, 4H, vinylic CH and aromatic nucleus. Mass spectrum shows *m/e* at 573, M^+-Bu.

Preparation of 17α-E-*iodovinyloestradiol-3-acetate* (1d). 17α-*E*-Tributylstannylvinyloestradiol-3-acetate (23 mg) was dissolved in glacial acetic acid (5 mL) containing sodium acetate (250 mg) and treated with sodium iodide (21 mg) and a 2:1 mixture of hydrogen peroxide and glacial acetic acid (10 mL). The mixture was stirred for 45 min; it was treated with an aqueous solution of sodium bisulphite (5 mL, 5%). The mixture was extracted with methylene chloride (2 × 25 mL). The combined extracts were washed with water until neutral, dried ($MgSO_4$) and the solvent removed to give the desired product. NMR (200 MHz): 0.7–2.4, 21H, m, steroidal envelope and CH_3-CO, 6.24–6.34, 1H, d, J = 14Hz, vinylic CH, 6.68–7.44, 4H, m, vinylic CH and aromatic nucleus. Mass spectrum shows *m/e* at 423, M^+-CH_3CO.

Preparation of 17α-*Z*-*iodovinyloestradiol-3-acetate* (1c). 17α-*Z*-Tributylstannylvinyloestradiol-3-acetate (20 mg) was similarly treated to give the desired *Z*-iodide. NMR (400 MHz): 0.7–2.4, 21H, m, steroidal envelope and CH_3-CO, 6.35–6.4, 1H, d, J = 8Hz, vinylic CH, 6.75–7.45, 4H, m, vinylic CH and aromatic nucleus.

Preparation of ^{125}I-*labelled compounds for administration to rats.* ^{125}I-Labelled 17α-*Z*- and 17α-*E*-iodovinyloestradiol-3-acetate (Fig. 1c and d, respectively) were prepared by oxidative iodination of the corresponding tributylstannyl compounds as described [14] and purified by HPLC. The fractions containing the desired material were combined and taken to dryness under a stream of nitrogen. The labelled compounds were characterized by co-chromatography with authentic cold compounds. The labelled compounds were taken up in 0.25 mL ethanol and diluted to 1 μCi per 0.1 mL with sterile saline for injection into rats. Deacetylation of the purified iodovinyloestradiol-3-acetates to yield 17α-*Z*- and 17α-*E*-iodovinyloestradiol (Fig. 1a and b) was carried out by a standard procedure [14]. No further purification of the products was required. It was administered to animals after neutralization with dilute hydrochloric acid and appropriate dilution in sterile saline. 16α-[^{125}I]Iodo oestradiol as received was dried under nitrogen reconstituted in 0.25 mL ethanol and appropriately diluted in sterile saline.

Tissue distribution studies in rats. Compounds (1 μCi) were administered to young mature female Sprague–Dawley rats by intravenous injection into the tail vein. Animals were killed by administration of a lethal dose of Sagatal and in order to facilitate the collection of blood a simultaneous dose of heparin (100 U) was administered i.p. in saline. Tissues were removed and the radioactivity determined by direct gamma counting.

RESULTS

The distribution of radioactivity between tissues after injection of ^{125}I-labelled 17α-*Z*- and 17α-*E*-iodovinyloestradiol and their corresponding 3-acetates to female rats (approximately 100 g body wt) is given in Table 1a, b, d and e. 16α-[^{125}I]Iodo oestradiol was purchased and results of tissue distribution studies presented in Table 1c. In a further experiment 16α-[^{125}I]iodo oestradiol was administered to larger animals (approximately 200 g body wt) and the results are given in Table 1f. The mean of results on two animals is reported, with the coefficient of variation of inter animal differences not exceeding 15% in any case. A similar distribution pattern was noted with each injected compound. The highest concentration of radioactivity (cpm/g tissue) was measured in the thyroid, followed by the liver followed by uterus and kidney, which was greater than that in lung or blood; the concentration in heart and spleen was similarly low. The concentration in the uterus was greater after injection of 17α-*Z*-iodovinyloestradiol, its 3-acetate or 16α-iodo oestradiol than after injection of 17α-*E*-iodovinyloestradiol or its 3-acetate.

The ratio of concentrations in uterus to blood in small animals are detailed in Table 2a–e from which it may be seen that similar values were obtained

Table 1. Continued.

(d)				(e)				(f)			
30	60	120	240	30	60	120	240	30	60	120	240
6	93	81	63	93	66	28	55	141	55	77	35
38	17	15	20	41	28	14	19	29	13	13	9.5
373	1124	1188	8642	533	3390	1952	9469	308	654	1152	4820
12	140	112	250	433	240	107	73	300	255	104	76
5.6	48	27	21	140	85	32	33	80	31	28	17
3.2	23	14	10	106	58	20	23	47	16	13	8.3
5.2	47	29	24	194	80	32	36	73	21	17	9.4
2.7	15	13	12	78	40	14	19	88	28	23	13

Table 2. Ratio of concentration of radioactivity in uterus: blood in young mature female rats (100 g) after i.v. injection of (a) 17α-Z-[^{125}I]iodovinyloestradiol, (b) 17α-E-[^{125}I]iodovinyloestradiol, (c) 16α-[^{125}I]iodo oestradiol, (d) 17α-Z-[^{125}I]-iodovinyloestradiol-3-acetate, (e) 17α-E-[^{125}I]iodovinyloestradiol-3-acetate and (f) 16α-[^{125}I]iodo oestradiol to 200 g rats (individual animal data are quoted)

Time post injection (min)	(a)		(b)		(c)		(d)		(e)		(f)	
30	1.1	1.3	1.7	1.4	9.5	3.6	1.5	1.6	2.3	1.6	3.8	5.4
60	2.4	2.1	2.8	2.0	5.5	8.4	6.0	5.1	1.9	3.1	6.4	2.5
120	2.6	2.5	1.9	1.8	4.4	5.9	5.1	6.5	1.8	2.3	4.7	7.3
240	3.0	4.0	2.4	2.0	4.5	3.7	3.1	3.1	3.0	2.7	3.3	2.9

Table 3. Iodine accumulation in the thyroid, % of injected dose up to 4 hr after i.v. injection of 17α-Z-[^{125}I]-iodovinyloestradiol (IVE) and 3-acetate (IVEA), 17α-E-[^{125}I]iodovinyloestradiol and 3-acetate, and 16α-[^{125}I]iodo oestradiol (Iodo E2) to 100 g and 200 g rats

Time (min)	30	60	120	240
17α-Z-[^{125}I]IVE	0.25	0.56	0.88	2.32
17α-Z-[^{125}I]IVEA	0.53	0.16	0.22	0.83
17α-E-[^{125}I]IVE	0.05	0.20	0.36	0.57
17α-E-[^{125}I]IVEA	0.08	0.30	0.25	1.15
16α-[^{125}I]Iodo E2 (100 g)	0.02	0.03	0.11	0.32
16α-[^{125}I]Iodo E2 (200 g)	0.05	0.11	0.16	0.39

Table 4. Ratio of concentration of radioactivity in uterus: blood in young mature female rats (100 g) after i.v. injection of 16α-[^{125}I]iodo oestradiol at various times after killing by (a) administration of a lethal dose of Sagatal or (b) by cervical dislocation (individual animal data are reported)

Time post injection (min)	(a)		(b)	
30	6.9	—	6.3	—
60	7.7	20.4	18.7	13.6
120	17.5	9.7	2.6	11.0
240	2.3	2.3	5.3	7.1

when either 17α-E-iodovinyloestradiol or its 3-acetate were injected. The ratio was greater after the injection of 17α-Z-iodovinyloestradiol-3-acetate than after the injection of the deacetylated compound. The ratio after injection of 16α-iodo oestradiol was similar to that after injection of 17α-Z-iodovinyloestradiol-3-acetate. In the larger animals (Table 2f) the ratios obtained were more variable but showed a trend to lower values than those in smaller animals (Table 2c).

Analysis of ether extracts of plasma by TLC yielded a single radioactive species after injection of 17α-E- or 17α-Z-iodovinyloestradiol or their 3-acetates. The radioactivity co-migrated with authentic 17α-E- or 17α-Z-iodovinyloestradiol, respectively, confirming that the 3-acetates are rapidly hydrolysed *in vivo* but that no further metabolism to other radioactive species occurs.

The highest concentration of radioactivity was noted in the thyroid irrespective of the compound injected. The radioactivity was resistant to extraction in ether indicating its inorganic nature. An estimate of the instability of the iodo compound injected was obtained from the radioactivity detected in the thyroid gland at various times post injection expressed as a percentage of the administered dose. These data are tabulated in Table 3. The results show that the concentration of radioactivity increases with time and that the Z-isomer, particularly as the deacetylated compound, is less stable than the E-isomer which was in turn less stable than the 16α-iodo compound.

In a further experiment 16α-[^{125}I]iodo oestradiol was administered to two groups of 100 g rats. At various times after injection animals received 100 U of heparin and were killed by cervical dislocation or by the administration of a lethal dose of Sagatal. There was no significant difference in the tissue distribution of radioactivity between the two groups which were also similar to those measured in the previous experiments. The results show no significant difference occasioned by the method of killing in the uterus to blood ratios (Table 4) but these were considerably higher than those measured in the previous set of experiments.

CONCLUSIONS

The chemical syntheses of 17α-E- and 17α-Z-tributylstannylvinyloestradiol-3-acetate were readily carried out. Iodination of these compounds was facile and excellent yields were obtained using radiolabelled sodium iodide. The iodination reaction using chloramine T and NaI is very rapid and efficient and can be accomplished within 30 min including HPLC purification. This is quicker and more efficient than the exchange reactions required for the preparation of the 16α-iodo oestradiols [12] and does not require the manipulation of the radioactive tracer prior to reaction. These are important considerations for the use of isotopes with a short half-life such as ^{123}I.

The uterine accumulation of the Z-isomer was superior to that of the E-isomer, confirming a previous report [12], and similar to the accumulation of 16α-iodo oestradiol. The uterus to blood ratios of radioactivity were higher after injection of 17α-Z-[^{125}I]iodovinyloestradiol-3-acetate than after injection of the corresponding deacetylated compound, due to the former's more rapid clearance from blood.

The results of uterine to blood ratios of radioactive concentrations were much lower using the 17α-E-iodovinyloestradiols and 16α-iodo oestradiol than we have previously measured [8, 14]. This set of experiments was carried out on Sprague–Dawley rats whereas previous experiments were carried out on Wistar rats. Both sets of experiments were carried out on animals at a similar age of development but Wistar rats are smaller than Sprague–Dawleys. In order to see if the discrepancy could be accounted for by a difference in size, 16α-[^{125}I]iodo oestradiol was administered to larger animals. The ratio between animals was very variable but a trend to lower ratios was seen in the heavier animals, suggesting that some of the difference in results may be attributable to weight differences between strains. A further difference between our previous experiments and those reported here was the method of killing of the animals. In the current experiments animals were killed by the injection of a lethal dose of Sagatal instead of cervical dislocation so that the thyroid glands could be removed. Therefore, we compared the method of killing on the tissue distribution of radioactivity after injection of 16α-[^{125}I]iodo oestradiol. No significant difference was detected. Interestingly, the uterus to blood ratios of radioactivity were more similar to our previously published data and suggest that the main reason for the between experiment differences is variation between animal groups.

Radioactivity accumulated in the thyroid with each of the compounds. The increase in concentration with time and the inorganic nature of the iodine points to extra thyroidal metabolism to liberate iodine which is then scavenged by the thyroid gland. Most radioactivity accumulated in the thyroid after injection of 17α-Z-[^{125}I]iodovinyloestradiol and least after injection of 16α-[^{125}I]iodo oestradiol. Acetylation at the three position increases the stability of the 17α-Z-iodide, which may be related to its more rapid clearance from the blood. These results indicate caution in the administration of these compounds labelled with radioisotopes to patients and highlight the need of blocking thyroid uptake.

In studies in patients, we have previously shown that the circulating radioactivity after injection of radiolabelled 16α-iodo oestradiol includes a more polar species than the injected compound, which we tentatively identified as 16α-iodo oestrone [8]. (We were surprised to note that Grill *et al.* [15] found 16α-iodo oestradiol stable to incubation with a post nuclear supernatant of human placenta, a tissue rich in 17β-dehydrogenase. The apparent discrepancy may be a reflection of entero hepatic recycling after intra venous administration.) Metabolism to a non-binding receptor species would be expected to reduce the specific target tissue accumulation of the injected compound. Substitution at the 17α position inhibits oxidation of the 17β-hydroxyl, as has been demonstrated after the i.v. injection of 17α-E-iodovinyloestradiol-3-acetate in humans [14]. It is reasonable, taking into consideration the stability of 17α-iodovinyloestradiols in the rat, to expect a similar stability with respect to the 17β-hydroxyl with the 17α-Z isomer in humans.

Taken together, chemical considerations and biological results support the view that the 17α-Z-iodovinyl substitution is the position of choice for an oestrogen receptor-seeking radiopharmaceutical and that further modification of the nucleus with the 3-acetate and the 11β-methoxy would yield the compound of choice for administration.

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