J. steroid Biochem. Vol. 35, No. 6, pp. 641–646, 1990
Printed in Great Britain. All rights reserved

0022-4731/90 $3.00 + 0.00
Copyright © 1990 Pergamon Press plc

THE SYNTHESIS OF E-17α-[^{131}I]IODO-VINYL OESTRADIOL AND EVALUATION OF ITS USE AS A RADIOTRACER FOR OESTROGEN RECEPTOR POSITIVE BREAST TUMOURS

ELIZABETH K. SYMES,* W. F. COULSON, R. DAS and J. H. SCURR[1]

Department of Biochemistry and [1]Department of Surgery, University College and Middlesex School of Medicine, University College London, Gower Street, London WC1E 6BT, England

(*Received* 19 *September* 1989)

Summary—17α-Iodo-vinyl oestradiol binds with high affinity to the oestrogen receptor, is metabolically stable and has been shown to accumulate in oestrogen sensitive tissues of rodents. We have synthesised this compound and its 3-acetate, labelled with carrier free ^{125}I and shown the accumulation of both compounds in rat uterus. 17α-Iodo-vinyl oestradiol-3-acetate was prepared labelled with carrier free ^{131}I and administered to twelve patients with suspected breast cancer, approximately 1 h before surgical removal of the primary tumour mass. At the time of surgery a sample of the tumour and a peripheral blood sample were taken. The ratio of radioactivity in the tumour to blood was determined. All tumours accumulated radioactivity and ratios ranged from 1.5:1 to 3.7:1. There was no correlation between the degree of accumulation and either cytosolic oestrogen or progesterone receptor concentration in the tumour. Analysis of blood revealed a single circulating species, 17α-iodo-vinyl oestradiol. The results show that 17α-iodo-vinyl oestradiol is metabolically stable and accumulates in breast tumours though this accumulation is not sufficient to permit imaging.

INTRODUCTION

A number of studies in rats have indicated that receptor binding halogenated oestrogens are accumulated in oestrogen target tissues [1–4]. By analogy these compounds should accumulate in oestrogen receptor positive breast tumours and suitably labelled with a gamma emitting isotope enable the imaging of these tissues by non-invasive external gamma scintigraphy. The ideal radiopharmaceutical should have high affinity and specificity for the oestrogen receptor, rapid clearance from and stability in the blood, and since selective uptake is mediated by the low capacity receptor must be prepared at very high specific activity [4–6]. Suitable radioisotopes include ^{131}I and ^{123}I.

We have recently prepared ^{131}I-labelled 16α-iodo oestradiol and tested this compound as a potential radiopharmaceutical for breast tumour imaging [7]. Images obtained in two receptor positive tumours were very faint and no images could be obtained in a further three receptor positive tumours. Considerable accumulation of the compound was detected in the gut and extensive conversion of the injected compound, to what we suspect was 16α-iodo oestrone, was demonstrated. The preparation of the ^{131}I-labelled compound was a slow and inefficient exchange reaction brought about by the heating of 16β-bromo oestradiol with ^{131}I-labelled sodium iodide in butanone. Heating for sixteen hours was required to obtain a reasonable yield of the ^{131}I-labelled 16α-iodo oestradiol. These conditions were therefore inappropriate for the synthesis of the compound labelled with an isotope of shorter half life such as ^{123}I ($t_{1/2}$ 12 h).

E-17α-iodo-vinyl oestradiol has been reported to bind avidly to the oestrogen receptor and accumulate in oestrogen dependent target tissues of the rat [3, 4]. This compound is metabolically stable. The iodine derivative can be very rapidly prepared in good yield from the boronic acid of ethynyl oestradiol with chloramine T and sodium iodide, so that this compound could be labelled with an isotope of short half life such as ^{123}I. This compound therefore offers advantages over 16α-iodo oestradiol as a receptor seeking radiopharmaceutical. We have prepared this compound labelled with ^{131}I and conducted preliminary tests for its accumulation in breast tumours.

EXPERIMENTAL

All reagents were of analar grade. Radioisotopes were purchased from Amersham International. The [^{1}H]NMR spectra were recorded on a Jeol-200 MHz instrument with tetramethylchlorosilane as the internal standard. The i.r. spectra were determined for

*Correspondence should be addressed to: Dr Elizabeth K. Symes, Division of Molecular Endocrinology, Hammersmith Hospital, Du Cane Road, London W12 0HS, England.

KBr discs using a Perkin Elmer 237 spectrophotometer. m.p.s were done on a Reichert melting point microscope. TLC was carried out on Kieselgel $60PF_{254+366}$. Tetrahydrofuran was distilled over lithium aluminium hydride before use. Petroleum ether refers to the fraction of boiling range 60–80°C.

Chemical synthesis of 17α-iodo-vinyl oestradiol and its 3-acetate

(17α, 20E)-3,17-dihydroxy-19-norpregna-1,3,5(10),-20-tetraene-21-yl boronic acid (1). Ethynyl oestradiol (300 mg, 0.1 mmol) was stirred with catecholborane (0.6 ml, 7.56 mmol) at 70°C for 4 h under nitrogen. After cooling to room temperature, water was added slowly and the reaction mixture left stirring overnight. The pale yellow solid formed was filtered and washed thoroughly with ice-cold water. The crude material was dried, dissolved in tetrahydrofuran, from which it was precipitated with petroleum ether (yield 37%, 125 mg). The material was not further purified as it was found to be unstable to heat and chromatography on Kieselgel.

i.r.$_{max}$ 3500–3090, 2960–2840, 1610 cm^{-1}; [^{1}H]NMR ($CD_3)_2SO$, δ 0.83 (s, 18-H_3), 4.44 (s, OH), 5.43 (d, J = 18 Hz, = CHB$(OH)_2$), 6.43 (broad s, 4-H), 6.49 (d, J = 9 Hz, 2-H), 650 (s, OH), 6.69 (d, J = 18 Hz, —CH = CHB $(OH)_2$), 7.01 (d, J = 9 Hz, 1-H), 7.50 (s, OH) and 8.94 (s, OH); R_f value on TLC 0.25 (chloroform : methanol; 95 : 5).

(17α, 20E)-3-(acetoxy)-17-hydroxy-19-norpregna-1,3,5(10),20-tetraene-21-yl boronic acid (2). The boronic acid **(1)** (20 mg, 0.06 mmol) was dissolved in pyridine (0.10 ml) and treated with acetic anhydride (0.10 ml) at room temperature overnight. The mixture was poured into iced water. The precipitate was filtered, washed with water and dried (21 mg). Due to the instability of the boronic acid group to heat and chromatographic procedures the material was not further purified.

m.p. of the crude product 200–210°C: i.r.$_{max}$ 3400–3220, 2920, 1750, 1620, 1370, 1230(sh), 1200 and 1010 cm^{-1}; [^{1}H]NMR ($(CD_3)_2SO$), δ0.84 (s, 18-H_3), 2.23 (s, 3-OAc), 4.48 (s, OH), 5.44 (d, J = 17.8 Hz, =CHB$(OH)_2$), 6.69 (d, J = 7.8 Hz, 1-H) and 7.53 (s, OH); R_f value on TLC 0.21 (petroleum ether : chloroform : methanol; 7 : 3 : 1).

(17α, 20E)-3-(acetoxy)-21-iodo-19-norpregna-1,3,5(10),20-tetraene-17-ol (3). Chloramine-T (2.92 mg, 0.01 nmol) was added to a mixture of **(2)** (4 mg, 0.01 mmol), tetrahydrofuran (0.08 ml), 0.066 M phosphate buffer (pH 7.0, 0.08 ml) and 1.0 M NaI (0.01 ml, 0.01 mmol). The reaction mixture was left at room temperature for 2 h in the dark with occasional agitation. The mixture was poured into water and the product extracted into ether. The ethereal layer was washed with 5% sodium thiosulphate, water and dried over magnesium sulphate. Evaporation of the ether under reduced pressure gave a pale yellow solid. The crude product was purified by TLC.

m.p. 102–107° (dec); i.r.$_{max}$ 3500–3400, 2920, 1745, 1595, 1365, 1200, 1010, 945 and 930 cm^{-1}; [^{1}H]NMR ($CDCl_3$), δ 0.92 (s, 18-H_3), 2.28 (s, 3-OAc), 6.30 (d, J = 14 Hz, HC = CHI), 6.70–6.90 (m, 2-H and 4-H), 6.78 (d, J = 14 Hz, HC = CHI), and 7.26 (d, J = 8 Hz, 1-H); R_f value on TLC 0.33 (petroleum ether : chloroform : methanol; 7 : 3 : 1).

(17α, 20E)-21-iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (4). The steroid **(3)** (9 mg, 0.017 mmol) was dissolved in tetrahydrofuran (0.9 ml) and ethanol (0.45 ml) and the solution cooled to 10°C. Sodium hydroxide (0.09 ml, 2.0 M) was added, causing a strong yellow colour to be discharged which persisted throughout the reaction. The temperature of the reaction was allowed to warm to room temperature and the reaction was monitored by TLC. The starting material had completely disappeared after 30 min. The mixture was poured into water, extracted with ether, dried over sodium sulphate and evaporated under reduced pressure. The crude product was purified by TLC.

m.p. 91–96°C (dec); i.r.$_{max}$ 3410–3340, 2910, 1610, 1450, 1230, 1000 and 950 cm^{-1}; [^{1}H]NMR ($CDCl_3$), δ 0.92 (s, 18-H_3), 6.29 (d, J = 14 Hz, HC = CHI), 6.50–6.70 (m, 2-H and 4-H), 6.78 (d, J = 14 Hz, HC = CHI) and 7.13 (d, J = 8 Hz, 1-H); R_f value on TLC 0.39 (toluene : ethyl acetate; 8 : 2) and 0.17 (dichloromethane : methanol; 99 : 1).

Preparation of 17α-[^{131}I]iodo-vinyl oestradiol-3-acetate for administration to patients

Steroid **(2)** (1 mg) was dissolved in tetrahydrofuran (0.5 ml). 50 μl were added to 20 μl tetrahydrofuran, 35 μl phosphate buffer (pH 7.5, 0.1 M), and 5 mCi Na[^{131}I]I as supplied in sodium hydroxide solution. To this mixture was added chloramine T (8 μl of solution of 100 mg in tetrahydrofuran/water, 50/50, v/v). After 20 min aqueous sodium metabisulphite was added (10 μl, 10 mg/ml, w/v). Radiolabelled 17α-iodo-vinyl oestradiol-3-acetate was purified from the starting material by HPLC. The crude reaction mixture was applied to a 25 cm × 4.6 mm reverse phase (C_{18}) column (S5 ODS2, Phase Separations Ltd, Queensferry, Clwyd, Wales). Tetrahydrofuran/water (1/1, v/v) was used as solvent at a flow rate of 1 ml/min. 1 ml fractions were collected and the desired product eluted 14–17 min. The identity of the radiolabelled material was confirmed by its co-chromatography with authentic unlabelled compound. The yield was 30% and the separation system excellent and reproducible.

The iodinated product was extracted into ether which was dried under a stream of N_2. The iodinated material was taken up in ethanol (0.5 ml). Just prior to injection 1.0 ml of a sterile solution of Tween 80 in water (1% v/v) was added and the mixture passed through a sterile filter (0.2 μ) of regenerated cellulose (Type 11607 Sartorius Instruments Ltd, Belmont, Surrey, England) which was then washed with 1.0 ml sterile normal saline. The radioactivity was diluted

Table 1. Tissue distribution of radioactivity (cpm × 10^{-3}/g tissue) after i.v. injection of (a) 17α-[^{125}I]iodo-vinyl oestradiol and (b) 17α-[^{125}I]iodo-vinyl oestradiol-3-acetate to immature female rats

Time (min)	Uterus	Liver	Kidney	Lung	Blood	Uterus/blood
(a) 17α-[^{125}I]iodo-vinyl oestradiol						
15	11.6	56.7	15.2	11.3	2.4	4.8
30	20.8	58.8	12.9	10.1	2.0	10.5
45	17.6	30.4	10.7	9.1	1.6	10.9
60	9.1	16.7	5.6	6.0	0.98	9.4
90	6.2	10.2	2.9	1.3	0.90	6.9
120	4.3	8.9	2.9	1.3	0.69	6.3
240	1.9	6.7	3.8	1.6	0.55	3.5
(b)17α-[^{125}I]iodo-vinyl oestradiol-3-acetate						
15	7.4	18.9	7.2	5.8	1.9	4.0
30	12.5	36.4	14.4	10.8	1.3	9.2
60	4.8	15.4	4.5	5.5	0.69	7.0
90	8.3	17.4	5.2	4.5	0.95	8.7
120	5.7	13.7	4.5	3.8	0.71	7.3
240	2.9	6.5	1.9	4.7	0.60	3.4

with sterile normal saline to a concentration of 0.25 mCi in 1 ml.

Distribution of 17α-iodo-vinyl oestradiol and its 3-acetate in female rats

^{125}I-labelled 17α-iodo-vinyl oestradiol-3-acetate was prepared as described above for the ^{131}I-labelled compound but for the substitution of 1 mCi Na [^{125}I]I. Deacetylation of this purified compound (as described for the unlabelled compound **(4)**, NaOH/ethanol, 0.5 h) yielded 17α[^{125}I]iodo-vinyl oestradiol. No further purification of the product is required. Therefore, after neutralisation with dilute hydrochloric acid the ethanolic solution was diluted with normal saline and sterilised. Labelled 17α-iodo-vinyl oestradiol or the 3-acetate (1 μCi) were administered to immature female Wistar rats. At various times after intravenous injection into the tail vein animals were killed by cervical dislocation, tissues were removed and the radioactivity in the tissues determined by direct gamma counting.

Clinical studies

Twelve patients undergoing surgery for the removal of a suspected primary breast tumour agreed to take part in the study. Each received 250 μCi of 17α[^{131}I]iodovinyl oestradiol-3-acetate by intravenous injection approximately 1 h before surgical removal of the tumour. The patients also took Lugols iodine (1 ml) to guard against possible liberation of radioactive iodine and its accumulation in the thyroid. Tumours removed at surgery were dissected to yield a peripheral portion (taken for pathological examination) and a central portion which was frozen as soon as possible in liquid nitrogen. At the same time as the tumour was removed, a peripheral blood sample was taken. The central tumour portion was taken for determination of radioactivity by direct gamma counting and for determination of cytosolic oestrogen and progesterone receptor concentrations. Plasma was extracted into ether and the etheral extract analysed by thin-layer chromatography on silica gel (developed in petroleum ether:chloroform:methanol, 7:3:1) to identify the nature of the circulating radioactive species.

RESULTS

17α-Iodo-vinyl oestradiol and its 3-acetate were prepared rapidly in good yield as described. Distribution studies of ^{125}I-labelled compounds in female rats (Table 1) indicated that both compounds were distributed similarly between tissues. Largest concentrations were noted in the liver followed by the uterus, kidney, and lung. Concentrations in brain, heart, spleen and blood were similarly low. The relative accumulation of the compounds in uterus against blood were of the order of 10:1 in the first hour and declined slowly thereafter.

In view of the similar behaviour of the two compounds in rats the 3-acetate was selected for labelling with ^{131}I and trial in patients as its synthesis involved few chemical manipulations. The results are given in Table 2. Pathological examination of the tumours removed at surgery confirmed infiltrating ductal carcinomas in all but patient 9 whose lesion was benign. Ratios of radioactivity in the tumour:blood indicated that all tumours, but not the benign lesion, accumulated the injected radioactivity. The degree of accumulation varied from 3.7:1 to 1.5:1. There was no obvious correlation between the degree of accumulation and the concentration of oestrogen receptor nor with the concentration of progesterone receptor in the breast lesion (see Fig. 1).

Analysis of the radioactivity in the plasma of each patient revealed a single circulating species which co-migrated with authentic 17α-iodo-vinyl oestradiol on silica gel chromatography.

DISCUSSION

The preparation of the boronic acid derivative of ethynyl oestradiol was readily accomplished. This compound was found to be unstable to heat and silica gel chromatography but could be partially purified by precipitation from solution in tetrahydrofuran with

Table 2. Accumulation of radioactivity and cytosolic receptor concentrations in breast tumours after i.v. injection of 250 μCi 17α-[^{131}I]iodo-vinyl oestradiol-3-acetate

Patient	Tumour: blood ratio radioactivity	E2R (fmol/mg	ProgR protein)	Age	Time post injection (min)
1	3.3	−ve	−ve	72	65
2	1.6	5	5	71	85
3	2.4	11	10	60	70
4	1.6	−ve	−ve	55	55
5	3.7	−ve	53	44	60
6	1.5	59	9	37	75
7	1.0–2.2	−ve	265/31	82	60
8	1.6–1.9	13	−ve	66	120
9	0.5	8	−ve	74	45
10	1.9	−ve	−ve	65	75
11	2.5–3.0	265	9	60	180
12	1.3–2.2	382	69	68	135

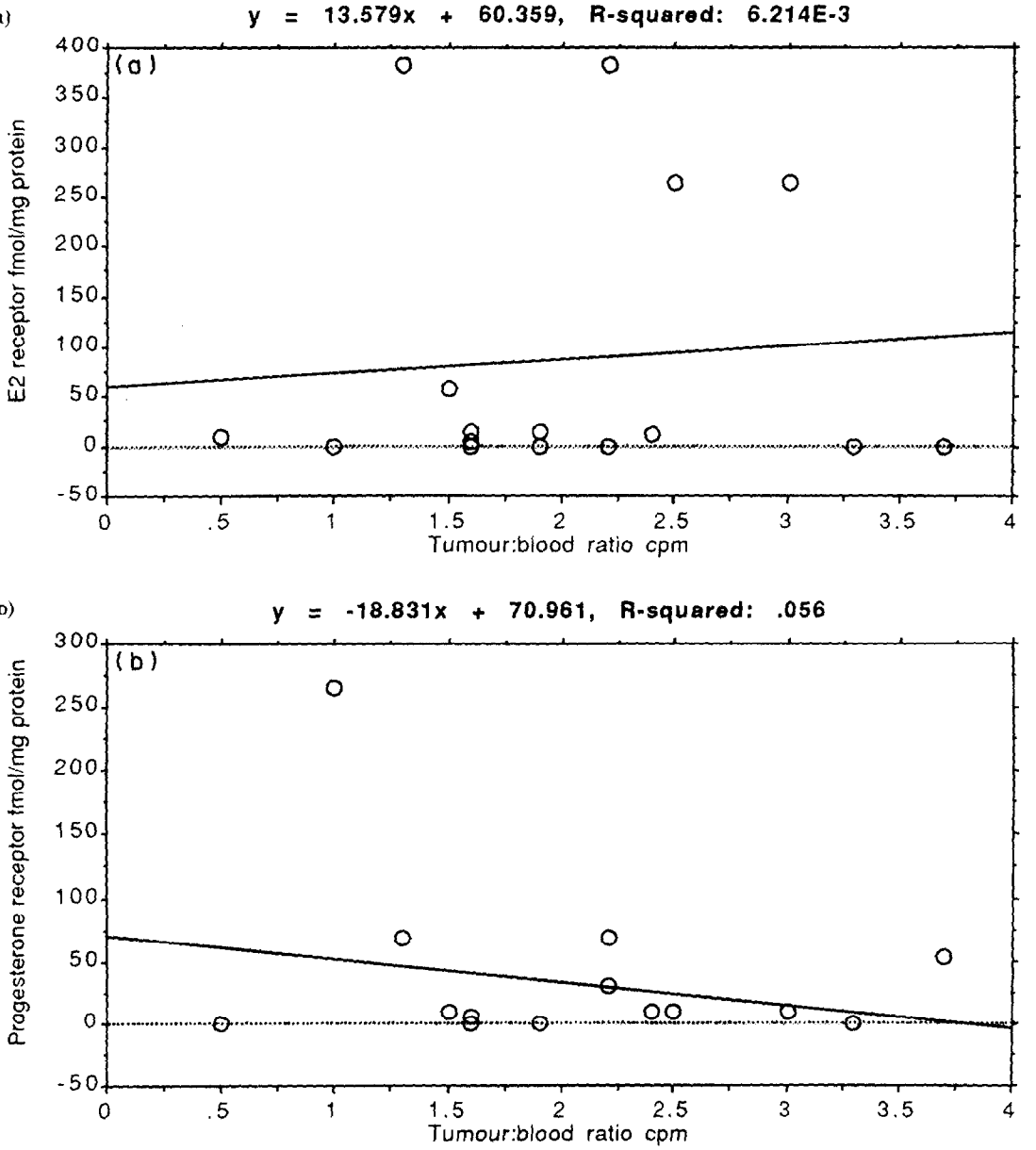

Fig. 1. Linear regression analysis between breast tumour: blood ratio of radioactivity after the injection of 250 μCi 17α-[^{131}I]iodo-vinyl oestradiol-3-acetate and tumour (a) oestrogen or (b) progesterone receptor concentration (fmol/mg protein).

petroleum ether. 17α-Iodo-vinyl oestradiol-3-acetate was prepared rapidly and in good yield by oxidative iodination of the boronic acid derivative of ethinyl oestradiol-3-acetate with cloramine T and NaI or Na[^{125}I]I or Na[^{131}I]I. The chemical synthesis we report is very similar to that described by Nakatsuka *et al.*[4] with the exception of the purification of the boronic acid derivatives. These authors were able to purify these compound on silica gel, whereas in our experience extensive breakdown took place during chromatography. We noted a decomposition temperature of 102–107°C similar to that reported [4]. However, deacetylation in our hands yielded a single compound as judged by thin-layer chromatography and [^{1}H]NMR with a decomposition temperature of 91–96°C. This is considerably lower than that reported [4]. This discrepancy is surprising but may be related to differences in the techniques employed for determining melting points i.e. rate of heating and length of exposure to high temperatures. The rapidity and efficiency of the iodination reaction would permit the preparation of 17α-iodo-vinyl oestradiol or its 3-acetate labelled with a short lived isotope such as ^{123}I.

Analysis of the patients' blood confirm the rapid hydrolysis of the injected acetate to and the metabolic stability of 17α-iodo-vinyl oestradiol. The results of the trial of this compound in patients confirm its accumulation in breast tumours but the degree of accumulation did not correlate with receptor status. Other studies in breast cancer patients with oestrogen receptor seeking radiopharmaceuticals [8, 9] report correlation between accumulation in the tumour and oestrogen receptor status. These studies were based on *in vivo* imaging. In our study we sampled the central portion of the tumour. This tissue may be poorly supplied with blood, therefore the radioactivity measured in this part of the tumour may be lower than that in the peripheral region. Receptor measurements may also be low if this tissue were necrotic. Four tumour specimens in this study were sufficiently large to permit multiple measurements of radioactive content to be made. In each case variability was encountered. As the receptor status was determined on only a small portion of the tissue, tumour heterogeneity may have contributed to the poor correlation. Endogeneous oestradiol is not likely to have contributed to the lack of correlation as most patients were post menopausal and one of the two premenopausal patient's tumour gave the best tissue to background ratio. In three patients the time elapsed between injection and administration was 2–3 times greater than usual (due to delays in theatre). Better accumulation may have occurred if the tissue had been analysed 60 min post injection. Studies in the rat and previous experience with 16α-iodo oestradiol suggest that 1 h post injection is optimal for accumulation.

Tissue to background ratios of at least 5:1 are required for imaging [10]. The maximum ratio obtained with this compound was 3.7:1 and ratios of >3:1 were obtained in two other tumours. These results are encouraging and perhaps could be improved with closer attention to the time of sampling. Further improvements might be brought about by the introduction of additional chemical modifications to 17α-iodo-vinyl oestradiol. Recent reports [4, 11] suggest that modifications in the 11β position improves accumulation in oestrogen sensitive tissues of rodents. Furthermore, Ali *et al.* [12] have demonstrated that the *Z* iodo-vinyl isomer is accumulated more avidly by oestrogen sensitive tissues of the rat than the *E* isomer. Clinical studies with these alternative compounds will be of interest.

Acknowledgements—This work was supported by a grant from the Middlesex Hospital Trustees. Karen Healy, of the Department of Clinical Research, Christie Hospital and Holt Radium Institute, Manchester, England kindly performed the receptor analyses.

REFERENCES

1. McElvany K. D., Carlson K. E., Welch M. J., Senderoff S. G., Katzenellenbogen J. A. and the Los Alamos Radioisotope Group: *In vivo* comparison of 16α-[^{77}Br]-bromoestradiol-17β and 16α-[^{125}I]iodoestradiol-17β. *J. Nucl. Med.* **33** (1982) 420–424.
2. Landvatter S. W., Kiesewetter D. O., Kilbourn M. R., Katzenellenbogen J. A. and Welch M. J.: (2*R**, 3*S**)-1-[^{18}F]Fluoro-2,3-bis(4-hydroxphenyl) pentane ([^{18}F]-fluoronor-hexestrol) a positron-emitting estrogen that shows highly-selective, receptor-mediated uptake by target tissues *in vivo*. *Life Sci.* **33** (1983) 1933–1938.
3. Hanson R. N., Seitz D. E. and Botarro J. C.: E-17α-[^{125}I]iodovinyl estradiol: an estrogen-receptor-seeking radiopharmaceutical. *J. Nucl. Med.* **23** (1982) 431–436.
4. Nakatsuka I., Ferreira N. L., Eckelman W. C., Francis B. E., Rzeszotarski W. J., Gibson R. E., Jagoda E. M. and Reba R. C.: Synthesis and evaluation of (17α, 20E)-21-[^{125}I]iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol and (17α, 20E)-21-[^{125}I]iodo-11β-methoxy-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (17α-iodovinyl estradiol derivatives) as high specific activity potential radiopharmaceuticals. *J. Med. Chem.* **27** (1984) 1287–1291.
5. McManaway M. E., Jagoda E. M., Kasid A., Eckelman W. C., Francis B. E., Larson S. M., Gibson R. E., Reba R. C. and Lippman M. E.: [^{125}I]17α-iodovinyl-11-β-methoxyestradiol interaction *in vivo* with estrogen receptors in hormone-dependent MCF-7 human breast cancer transfected with the r-ras^H oncogene. *Cancer Res.* **47** (1987) 2945–2949.
6. Katzenellenbogen J. A., Carlson K. E. and Katzenellenbogen B. S.: Facile geometric isomerization of phenolic non-steroidal estrogens and antiestrogens: limitations to the interpretation of experiments characterising the activity of individual isomers. *J. Steroid Biochem.* **22** (1985) 589–596.
7. Symes E. K., Coulson W. F. and Ralphs D. N. L.: The synthesis of 16α-[^{131}I]iodo-oestradiol and evaluation of its use as a radiotracer for oestrogen receptor positive breast tumours. *J. Steroid Biochem.* **22** (1985) 155–160.
8. McElvany K. D., Katzenellenbogen J. A., Schaffer K. E., Siegel B. A., Senderoff S. G., Welch M. J. and the Los Alamos Medical Radioisotope Group: 16α-[^{77}Br]-bromoestradiol: dosimetry and preliminary clinical studies. *J. Nucl. Med.* **23** (1982) 425–430.

9. Katzenellenbogen J. A., Pomper M. G., Mathias C. J., Broklly H van, Brodock J. W., Mintun M. A. and Welch M. J.: Flourine-18 labelled estrogens as imaging agents for estrogen receptor positive breast tumours. *8th Int. Cong. of Endocrinology*, Kyoto, Japan (1988) Abstr. No. 16-19-131.
10. Ell P. J. and Khan O.: Emission computerised tomography: clinical applications. *Seminars in Nuclear Medicine XI* (1981) pp. 50–60.
11. Hanson R. N., Franke L. A. and Kaplan M. L.: Synthesis and evaluation of (17α, 20E)21-[^{125}I]iodo-11-substituted-19-norpregna-1,3,5(10),20-tetraene-3,17diols: the influence of 11-stereochemistry on tissue distribution of radioiodinated estrogens. *Nucl. Med. Biol.* **16** (1989) 3–9. *Int. J. Radiot. Appl. Instrum.* Part B.
12. Ali H., Rousseau J., Ghaffori M. A. and Van Lier J. E.: Synthesis, receptor binding and tissue distribution of (17α, 20E)- and (17α, 20Z)-21-[^{125}I]iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol. *J. Med. Chem.* **31** (1988) 1946–1950.