dose producing 50% reduction of the control isoprenaline response) and 95% confidence limits were calculated from the log dose–response relationship established by linear regression.[19] Statistical analysis of the results showed that the 95% confidence limits for the ED_{50} values averaged 30% (standard deviation 14%).

β-Adrenoceptor agonism was measured by the method of Barrett and Carter,[20] in rats anesthetized with pentobarbitone sodium (75 mg/kg ip) and treated 20–24 h previously with reserpine (5 mg/kg ip). Single doses of test compound were administered into the tail vein, and up to 20 animals were used, depending on the activity. Blood pressure and heart rate were recorded as above. A dose–response relationship was established, and ED_{30} value (defined as the dose producing a 30 beats/min increase in heart rate) and 95% confidence limits were calculated as above. Statistical analysis of the results showed that the 95% confidence limits for the ED_{30} values averaged 60% (standard deviation 37%).

Acknowledgment. We thank the Physical Methods Department for the elemental analyses and IR and NMR spectra and Dr. A. Krohn for the computer modeling studies.

Registry No. **1**, 80200-45-9; **2**, 80200-39-1; **3**, 80200-61-9; **4**, 80200-65-3; **5**, 80200-42-6; **5** (free base), 88670-84-2; **6**, 80200-66-4; **6** (free base), 88670-85-3; **7**, 80200-33-5; **7** (free base), 88670-86-4; **8**, 88670-67-1; **9**, 80200-41-5; **10**, 80200-58-4; **11**, 80200-26-6; **12**, 80200-68-6; **13**, 80200-24-4; **14**, 80200-30-2; **15**, 80200-37-9; **16**, 80200-35-7; **17**, 80200-54-0; **17** (free base), 88670-87-5; **18**, 80200-53-9; **19**, 80200-49-3; **20**, 80200-32-4; **21**, 80200-27-7; **21** (free base), 88670-88-6; **22**, 80200-28-8; **22** (free base), 88670-89-7; **23**, 80200-46-0; **23** (free base), 88670-90-0; **24**, 80200-47-1; **24** (free base), 88670-91-1; **25**, 80200-56-2; **26**, 88670-69-3; **27a**, 41833-17-4; **27b**, 80200-09-5; **28**, 61439-60-9; **29**, 3351-59-5; **30**, 80199-92-4; **31a**, 80199-93-5; **31b**, 88670-73-9; **31c**, 88670-74-0; **32a**, 88670-75-1; **32b**, 88670-76-2; **32c**, 88670-77-3; **32d**, 80199-91-3; **32e**, 88670-78-4; **32f**, 80200-69-7; **32g**, 80200-21-1; **32h**, 88670-79-5; **32i**, 88670-80-8; **32j**, 80200-22-2; **32k**, 88670-81-9; **32l**, 80199-94-6; **33a**, 88670-82-0; **33b**, 88670-83-1; **34a**, 80200-06-2; **34b**, 80200-08-4; **34c**, 80200-07-3; **35a**, 80199-99-1; **35b**, 80200-00-6; **35c**, 80200-01-7; **35d**, 80199-98-0; **35e**, 80200-05-1; **35f**, 80200-04-0; **35g**, 80200-15-3; **35h**, 80200-16-4; **35i**, 88670-72-8; **35j**, 80200-10-8; **35k**, 80200-11-9; **36a**, 80200-03-9; **36b**, 80200-02-8; **40**·HCl, 88070-70-6; **41**·HCl, 73901-67-4; **42**, 80200-20-0; **43a**, 80200-19-7; **43b**, 80200-14-2; **44**, 78910-04-0; **45**·HCl, 88670-71-7; **46a**, 80199-96-8; **46b**, 80199-97-9; **47a**, 80200-18-6; **47b**, 80200-17-5; **48**, 80199-86-6; **49**, 80199-87-7; 4-mercaptophenol, 637-89-8; 2-(isopropylamino)-3-(4-hydroxyphenoxy)-2-propanol, 16656-05-6; 1*H*-1,2,4-triazole, 288-88-0; 4-chloro-1*H*-pyrazole, 15878-00-9; 4-phenyl-1*H*-pyrazole, 10199-68-5; 2*H*-1,2,3-triazole, 288-35-7; 1*H*-benzotriazole, 95-14-7; formalin, 50-00-0; 1-(2-hydroxyethyl)pyrazole, 6314-23-4; 4-hydroxybenzyl alcohol, 623-05-2; imidazole, 288-32-4; 1*H*-pyrazole, 288-13-1; 1*H*-benzimidazole, 51-17-2; 1*H*-indazole, 271-44-3; 2*H*-benzotriazole, 273-02-9; 4-(benzyloxy)phenol, 103-16-2; 1-(dimethylamino)-3-[4-(benzyloxy)phenyl]propan-3-one, 51345-76-7.

(19) Davies, O. L.; Goldsmith, P. L. Eds. "Statistical Methods in Research and Production"; Oliver and Boyd: Edinburgh, 1972; Chapter 7, p 178.
(20) Barrett, A. M.; Carter, J. *Br. J. Pharmacol.* **1970**, *40*, 373.

Synthesis, Receptor Binding, and Target-Tissue Uptake of Carbon-11 Labeled Carbamate Derivatives of Estradiol and Hexestrol

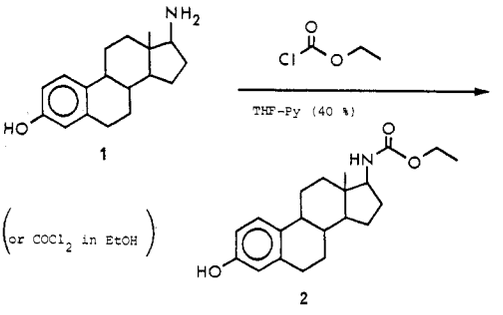
René Ouellet,† Jacques Rousseau,† Nicole Brasseur,† Johan E. van Lier,*,† Mirko Diksic,‡ and Gerrit Westera†,§

MRC Group in the Radiation Sciences, University of Sherbrooke Medical Center, Sherbrooke, Quebec, Canada, J1H 5N4, and Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4. *Received July 5, 1983*

The reaction of ethyl chloroformate with amino compounds has been evaluated as a simple route to carbon-11 labeling of steroid hormone–receptor-based imaging agents. Both a 17β-amino analogue of estradiol and an aminoethyl derivative of the nonsteroidal estrogen hexestrol with potential affinity for the estrogen receptor were studied. The unlabeled carbamate derivatives of the amino estrogens were prepared by standard methods, and the ^{11}C-labeled analogues were synthesized from [^{11}C]ethyl chloroformate, generated by purging ethanol with [^{11}C]phosgene. Both carbamates showed weak in vitro binding affinity for the estrogen receptor, and only the ^{11}C-labeled hexestrol exhibited a small but significant estrogen-responsive uterus uptake in immature rats.

With the aim to develop radiopharmaceuticals for imaging estrogen receptor positive human breast tumors, a variety of steroidal and nonsteroidal estrogens labeled with γ-emitting radioisotopes have been prepared over the past few years.[1-11] Most of these analogues were labeled with radioisotopes of iodine. Although various vinyl and aryl iodides possess reasonable metabolic stability,[10] aliphatic iodides exhibit poor in vivo stability with the attendant risk for high radioactivity uptake in the thyroid.[11] Accordingly, bromine-77 has recently been favored as the radiohalogen of choice.[8,9,11] In addition to radiohalogens and mercury-203,[12] the positron-emitting carbon-11 radionuclide has also been used for the labeling of steroid analogues.[13,14] The short 20-min half-life of carbon-11 requires that ^{11}C-labeled estrogens have rapid in vivo target localization with rapid clearance from nontarget tissues.

Scheme I



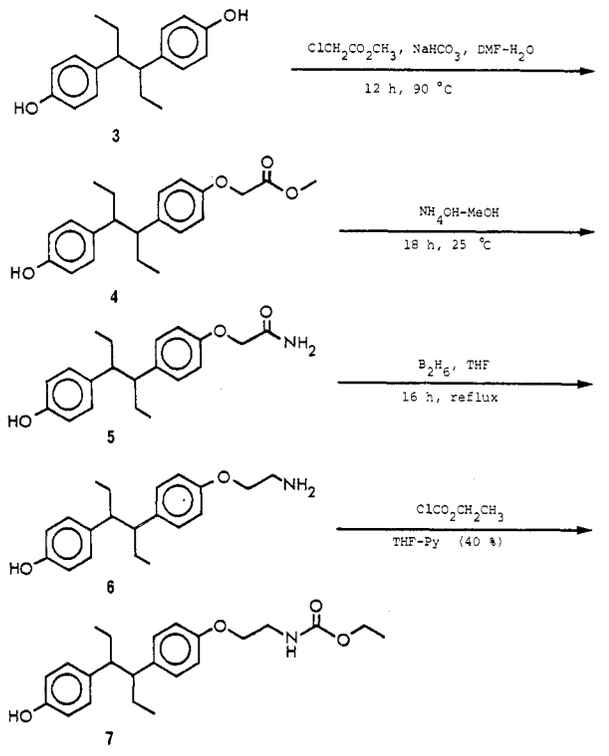
In addition, fast, convenient synthetic methods are required for the preparation of ^{11}C-labeled estrogens.

†MRC Group in the Radiation Sciences.
‡Montreal Neurological Institute.
§On leave from the Vrije Universiteit, Amsterdam, The Netherlands.

(1) Komai, T.; Eckelman, W. C.; Johnsonbaugh, R. E.; Mazaitis, A.; Kubota, H.; Reba, R. C. *J. Nucl. Med.* **1977**, *18*, 360.
(2) Arunachalam, T.; Longcope, C.; Caspi, E. *J. Biol. Chem.* **1979**, *254*, 5900.
(3) Hochberg, R. B. *Science* **1979**, *205*, 1138.

0022-2623/84/1827-0509$01.50/0 © 1984 American Chemical Society

Scheme II



^{11}C-labeled phosgene has recently been used as a synthon for the preparation of ^{11}C-labeled radiopharmaceuticals,[15-18] and we now have explored its potential for labeling estrogens. Two estrogenic carbamates were chosen as model compounds, and their syntheses are described. Since hexestrol ethers exhibit weak binding affinity for the estrogen receptor,[19] it was projected that the analogous carbamate derivative might have similar properties. The competitive in vitro binding of the nonradiolabeled carbamates and the intermediate hexestrol ether derivatives with the estrogen receptor was evaluated. The corresponding [^{11}C]carbamates were studied for in vivo receptor-mediated uptake in the rat uterus.

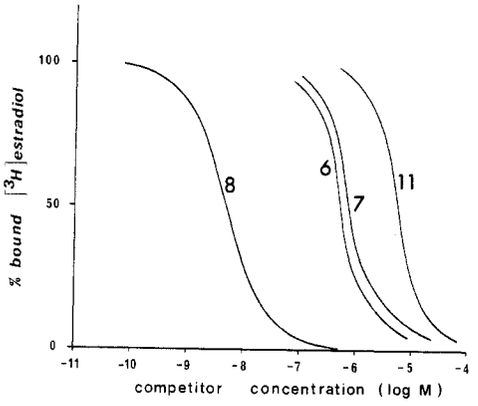
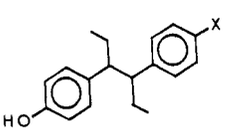
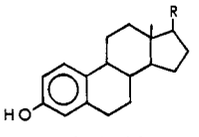


Figure 1. Competition between 10^{-9} M [^{3}H]estradiol and 10^{-5} to 10^{-11} M unlabeled estradiol (**8**) and hexestrol derivatives **6**, **7**, and **11**. The concentration required for 50% competition was used to calculate RBA values (see Table I).

Table I. Estrogen Receptor Binding. Affinity of Estradiol and Hexestrol Derivatives[a]



3-7, 11



1, 2, 8-10

no.	R or X	RBA[b]
8	OH	100
9	=O	23 (25-50)[c]
10	=NOH	12
1	NH_2	0.50
2	$NHCO_2CH_2CH_3$	0.32
3	OH	30 (100)[c]
4	$OCH_2CO_2CH_3$	0.15
5	OCH_2CONH_2	0.76
6	$OCH_2CH_2NH_2$	0.89
7	$OCH_2CH_2NHCO_2CH_2CH_3$	0.44
11	OCH_2CO_2H	0.06 (0.15)[d]

[a] The binding affinity was determined relative to that of [^{3}H]estradiol by a competition binding assay.[21] [b] The relative binding affinity (RBA) is 100 times the ratio between the competitor and unlabeled estradiol concentrations required for 50% competition.[22] Accordingly, by definition, the RBA of estradiol equals 100. [c] This datum is from ref 22. [d] The datum is calculated from the RCA value given in ref 19.

Chemistry. The starting material for carbamate **2**, the corresponding amine **1**, was prepared from estrone via estrone oxime by reduction with sodium in propanol (Scheme I).[20] The amine **6** was synthesized by converting hexestrol **3** into the methyl ester **4**, which was processed to give the amide **5**. The amine **5** was reduced to the amine **6** with diborane in refluxing THF (Scheme II). The carbamates **2** and **7** were then prepared by reacting the amines **1** and **6** with ethyl chloroformate (Schemes I and II). The carbamates labeled with carbon-11 ([^{11}C]**2** and [^{11}C]**7**) were made by bubbling [^{11}C]phosgene in ethanol, after which the amines **1** and **6** were added. The [^{11}C]-phosgene was prepared at a modest specific activity of about 40 Ci/mmol by the UV-induced reaction of ^{11}CO with Cl_2.[18] The unlabeled products were identified by

(4) Mazaitis, J. K.; Gibson, R. E.; Komai, T.; Eckelman, W. C.; Francis, B.; Reba, R. C. *J. Nucl. Med.* **1980**, *21*, 142.
(5) Katzenellenbogen, J. A.; Carlson, K. E.; Heiman, D. F.; Goswami, R. *J. Nucl. Med.* **1980**, *21*, 550.
(6) Longcope, C.; Arunachalam, T.; Rafkind, I.; Caspi, E. J. *Steroid Biochem.* **1981**, *14*, 261.
(7) Gatley, S. J.; Shaughnessy, W. J.; Inhorn, L.; Lieberman, L. M. *J. Nucl. Med.* **1981**, *22*, 459.
(8) Katzenellenbogen, J. A.; Senderoff, S. G.; McElvany, K. D.; O'Brien. Jr., H. A.; Welch, M. J. *J. Nucl. Med.* **1981**, *22*, 42.
(9) Gibson, R. E.; Eckelman, W. C.; Francis, B.; O'Brien, Jr., H. A.; Mazaitis, J. K.; Wilbur, S.; Reba, R. C. *Int. J. Nucl. Med. Biol.* **1982**, *9*, 245.
(10) Hanson, R. N.; Seitz, D. E.; Botarro, J. C. *J. Nucl. Med.* **1982**, *23*, 431.
(11) Landvatter, S. W.; Katzenellenbogen, J. A.; McElvany, K. D.; Welch, M. J. *J. Med. Chem.* **1982**, *25*, 1307.
(12) Shani, J.; Lieberman, L. M.; Samuni, A.; Schlesinger, T.; Cais, M. *Int. J. Nucl. Med. Biol.* **1982**, *9*, 251.
(13) Berger, G.; Maziere, M.; Prenant, C.; Sastre, J.; Comar, D. *Int. J. Appl. Radiat. Isot.* **1981**, *32*, 811.
(14) Feenstra, A.; Nolten, G. M. J.; Vaalburg, W.; Reiffers, S.; Woldring, M. G. *J. Nucl. Med.* **1982**, *23*, 599.
(15) Roeda, D.; Crouzel, C.; van Zanten, B. *Radiochem. Radioanal. Lett.* **1978**, *33*, 175.
(16) Roeda, D.; Crouzel, C.; van der Jagt, P. J.; van Zanten, B.; Comar, D. *Int. J. Appl. Radiat. Isot.* **1980**, *31*, 549.
(17) Roeda, D.; Westera, G. *Int. J. Appl. Radiat. Isot.* **1981**, *32*, 931.
(18) Diksic, M.; Jolly, D.; Farrokhzad, S. *Int. J. Nucl. Med. Biol.* **1982**, *9*, 283.
(19) Katzenellenbogen, J. A.; McGorrin, R. J.; Tatee, T.; Kempton, R. J.; Carlson, K. E.; Kinder, D. H. *J. Med. Chem.* **1981**, *24*, 435.
(20) Wheeler, O. H.; Reyes-Zamora C. *Can. J. Chem.* **1969**, *47*, 160.

Table II. Organ Uptake of [^{11}C]2 and [^{11}C]7[a]

tissue	control % inject. dose/g	control SD	with estradiol % inject. dose/g	with estradiol SD
	[^{11}C]2			
blood	0.9	0.9	1.3	0.7
lung	3.1	2.6	5.1	3.0
liver	9.4	9.6	12.8	8.5
spleen	2.3	1.5	3.6	2.0
ovaries	6.5	5.4	7.4	3.7
uterus	4.5	2.6	7.8	2.9
adrenals	14.3	7.8	21.6	16.7
kidneys	4.2	4.4	6.1	4.4
muscle	1.6	1.7	3.2	1.7
	[^{11}C]7			
blood	0.81	0.46	1.3	1.9
lung	2.1	1.6	2.1	1.3
liver	8.5	6.4	7.8	4.6
spleen	2.4	2.0	1.6	1.0
ovaries	2.5	1.3	3.5	4.0
uterus	5.0	3.8	6.2	9.8
adrenals	6.5	3.7	5.3	3.8
kidneys	2.9	1.6	5.1	6.8
muscle	1.1	0.78	1.3	1.0

[a] Organ uptake (percent of injected dose per gram, normalized for animals of 100 g) 10 min after injection of the ^{11}C-labeled derivatives of estradiol ([^{11}C]2) and hexestrol ([^{11}C]7) in the presence or absence of coinjected estradiol (mean plus or minus standard deviation for five rats). Comparison between estradiol-treated animals and untreated animals by means of the Student's t test for matched pairs revealed no significant difference in the ^{11}C distribution pattern for **2** or **7**.

standard spectroscopic techniques, which included IR, ^{1}H NMR, and MS. The ^{11}C-labeled products **2** and **7** were cochromatographed with the unlabeled standards to confirm identity.

The specific activities of [^{11}C]2 and [^{11}C]7 were determined by coinjecting a known amount of the authentic samples onto the HPLC column. When used for animal experiments the ^{11}C-labeled product was brought to dryness after HPLC purification, redissolved in ethanol, and diluted in saline with required carrier in 0.2–0.3 mL.

Biological Properties. The affinity of the nonradiolabeled compounds for estrogen receptors was determined by competition studies with [^{3}H]estradiol.[21] Radioligand binding to calf uterine cytosol was measured by charcoal absorption, and the percentage bound radioligand was plotted against the concentration of unlabeled test substance. Competitors were tested at seven concentrations to give a semilog plot from which the molar concentration of unlabeled estradiol, hexestrol, or estrogen competitor that reduced radioligand binding by 50% was determined. The results from these assays are shown in Figure 1, and the relative binding affinities (RBA values)[22] derived from the radioligand displacement curves are summarized in Table I. Compared to the parent compounds hexestrol and estradiol, both carbamate derivatives **2** and **7** exhibit only weak binding affinity. In the hexestrol series, the carboxy compound **11** has the lowest RBA value, whereas the amino derivative **6** shows about a tenfold higher binding affinity. In the estradiol analogue series the 17-oxime **10** shows the highest affinity, whereas the amino derivative **1** binds in the same order of magnitude as the analogous hexestrol derivative (Table I).

Table III. Tissue to Blood Ratios of [^{11}C]2 and [^{11}C]7[a]

tissue	control tissue/blood ratio	control SD	with estradiol tissue/blood ratio	with estradiol SD
	[^{11}C]2			
lung	3.7	0.6	3.7	0.2
liver	9.1	0.5	9.1	1.2
spleen	2.8	0.4	2.7	0.5
ovaries	9.4	5.0	6.0	1.1
uterus	5.5	1.6	6.2	0.6
adrenals	18.0	4.1	14.9	3.0
kidneys	4.8	0.7	4.3	0.4
muscle	1.4	0.3	1.8	0.3
	[^{11}C]7			
lung	2.4	0.8	2.5	1.3
liver	9.0	3.2	9.4	3.9
spleen	2.5	1.1	1.9	0.5
ovaries	3.0	0.8	2.8	0.6
uterus	6.3	2.1	3.1	1.0
adrenals	8.0	0.6	5.4	3.5
kidneys	3.7	0.5	3.3	0.3
muscle	1.2	0.5	1.3	0.4

[a] Tissue to blood ratios 10 min after injection of ^{11}C-labeled derivatives of estradiol ([^{11}C]2 and hexestrol ([^{11}C]7 with and without coinjected estradiol in immature female rats (mean plus or minus standard deviation for five rats). A significant difference between estradiol-treated animals and untreated animals was only observed in the case of [^{11}C]7 for uterine to blood ratios ($p < 0.04$, Student's t test for matched pairs).

The results from the in vivo tissue distribution studies with the ^{11}C-labeled carbamates **2** and **7** were in accordance with the observed low binding affinities of the corresponding nonradiolabeled substances. In these studies, 50-g, 3–4 week old immature female Fisher rats that were injected via the caudal vein with the HPLC-purified ^{11}C-labeled substances were used. In order to show receptor-mediated uptake in the uterus, a separate group of animals was simultaneously injected with the tracer and with 18 μg of unlabeled estradiol.[11] Doses in this range block over 95% of the available estrogen receptor sites. Animals were sacrificed 10 min after injection with the radiotracer. The activity in the various organs is presented in Table II, indicating little specific uterine uptake of either [^{11}C]2 or [^{11}C]7. There appears to be significant accumulation of [^{11}C]2 in the adrenals (Table II). In addition, [^{11}C]7 showed a significant difference in the uterus/blood ratio between estradiol-treated animals and nontreated animals, whereas [^{11}C]2 uptake ratios were not affected by unlabeled estradiol (Table III).

Discussion

In this study we report the synthesis of some new derivatives of estradiol and hexestrol, including the two new ^{11}C-labeled carbamates **2** and **7**. The specific activities of the ^{11}C-labeled carbamates varied between 1 and 10 Ci/mmol, which are within a specific activity range similar to those reported for other ^{11}C-labeled products.[14,23] These specific activities are two to three orders of magnitude lower than those obtained for the 16α-[^{77}Br]bromo-11β-methoxy-17β-estradiol, which is presently one of the most promising estrogen receptor binding radiopharmaceuticals.[11] The specific activity of our ^{11}C-labeled estrogens is limited by the specific activity of the [^{11}C]phosgene, which in turn appears to depend mainly on the purity of

(21) McGuire, W. L.; De La Garza, M. *J. Clin. Endocrinol. Metab.* **1973**, *37*, 986.
(22) Raynaud, J. P.; Ojasoo, T.; Bouton, M.-M.; Philibert, D. In *Drug Des.* **1979**, 169–214.
(23) Wolf, A. P.; Redvanly, C. S. *Int. J. Appl. Radiat. Isot.* **1977**, *28*, 29.

the chlorine gas used to react with the ^{11}CO.[18] The latter is formed with a high specific activity of at least 10^4Ci/mmol, which decreases almost 10^3-fold to 40 Ci/mmol upon formation of the [^{11}C]phosgene.[18] Thus, higher specific activities of our ^{11}C-labeled estrogens can only be achieved by more stringent control of carrier introduction during [^{11}C]phosgene formation. Since the present specific activities and radiochemical yields were sufficient for in vivo uterine uptake studies, no effort was made to improve these values at this time.

The effect of structural variations on the affinity of these analogues for the estrogen receptor was determined by in vitro assay. The affinity of our estradiol derivatives for the estrogen receptor diminishes progressively upon substitution of the 17β-hydroxyl group for a keto (**9**), oxime (**10**), and 17β-amino group (**1**), whereas conversion of the latter to the carbamate **2** did not result in further loss of binding properties (Table I). These observations confirm earlier reports that the affinity of estradiol-bearing substituents on the 17β-oxygen is sensitive both to the size and the polarity of the substituent.[24] In the hexestrol series, a similar loss of receptor-binding affinity is observed for the ester derivatives **4–7** (Table I). The polar carboxy derivative **11** exhibits the lowest RBA value of all estrogen derivatives under study, which is in accordance with published data.[24]

Receptor-mediated tissue-localizing properties of our ^{11}C-labeled compounds was evaluated by in vivo tests. Previous studies suggested that maximum uterine uptake is achieved within 15 min;[7] accordingly, we selected only the 10-min time point for our animal studies. Although longer periods would allow activity to clear from blood and nontarget tissues to yield a possibly higher target to nontarget ratio, the short physical half-life of ^{11}C would render prolonged studies only marginal usefull. No significant differences were found between the tissue uptake in animals treated and untreated with unlabeled estradiol (Table II). This is not surprising in view of the rather low RBA values for these compounds. However, in the case of the hexestrol derivative [^{11}C]**7**, a slight but significant decrease was observed in the uterus/blood ratio upon coinjection of estradiol with the labeled agent (Table III). This effect is probably more likely a result of a persistant higher blood concentration of the tracer rather than higher uterine uptake. Together with the relative high adrenal retention of [^{11}C]**2**, these observations might be explained by differences in metabolic turnover rates between [^{11}C]**7** and [^{11}C]**2**.

In conclusion, we have shown that it is possible to use [^{11}C]phosgene in ethanol for the labeling of amine-substituted estrogens. The corresponding carbamates are formed readily at specific activities of 1–10 Ci/mmol, and they are conveniently separated from the parent compounds by HPLC. Derivatization of the amine group did not significantly alter the receptor-binding properties of the parent compounds. Thus, although the application of the present carbamates is limited, the method appears to be a valid approach for the production of receptor-binding ^{11}C radiopharmaceuticals if applied to amines with high affinity for the estrogen receptor.

Experimental Section

General Procedures. Starting materials and reagents for the syntheses were obtained commercially and were of the highest chemical grade available. Carbon-11 (physical half-life 20 min) was produced by the Japan Steel Works medical cyclotron of the Montreal Neurological Institute, McGill University, via the (p,α) reaction on nitrogen and processed to [^{11}C]phosgene by reacting ^{11}CO with chlorine gas in a quartz tube facing a 275-W CGE suntanner bulb.[18] The thin-layer chromatography (TLC) was conducted on 0.25-mm thick Brinkman silica gel 60 F-254 plates. Merck silica gel PF-254 (2 mm) on glass plates was used for preparative TLC. The following two solvent systems were routinely used: system I, 1:2 EtOAc/hexane; system II, 1:9 *i*-PrOH/CH_2Cl_2. Compounds were located on TLC plates by their fluorescence under a UV lamp or by their color response upon spraying with H_2SO_4/EtOH and heating at 120 °C. Chromatograms containing ^{11}C-labeled products were scanned for radioactivity with an ionization chamber detector. The high-pressure liquid chromatography (HPLC) was performed on a Whatman Partisil PXS 10/25 PAC column with 7:93 *i*-PrOH/heptane (or hexane) at a flow rate of 2 mL/min. Compounds were detected by their absorption at 254 or 280 nm and, where appropriate, by their γ radiation, which was detected via a sodium iodide detector. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Spectrospin 90-MHz apparatus with $CDCl_3$ as the solvent. Chemical shifts are reported in δ relative to Me_4Si as an internal standard. The infrared (IR) spectra were taken with a Perkin-Elmer Model 457 spectrophotometer equipped with a beam condenser. The high- and low-resolution mass spectra (MS, HRMS) were determined with a V9 micromass Model ZAB-1F apparatus at 70-eV ionization voltage, of the Chemistry Department, University of Sherbrooke. Combustion analyses were performed by Microanalysis Laboratories Ltd., Markham, Canada.

***N*-Carbethoxy-3-hydroxyestra-1,3,5(10)-trien-17β-ylamine (2).** A mixture of the amine **1** (Wheeler et al.[20]) (0.1 g, 0.37 mmol) in tetrahydrofuran (3 mL), pyridine (2 mL), and ethyl chloroformate (40 mg, 0.37 mmol) was left at 25 °C for 5 min, evaporated, dissolved in EtOAc containing 10% HCl, washed with water, and dried ($MgSO_4$). The crude mixture was purified by preparative TLC in system I to give the carbamate **2** (30 mg): mp 103–104 °C (from ether–petroleum ether, bp 68–95 °C); TLC R_f (EtOAc) 0.50, R_f (system I) 0.27, R_f (system II) 0.67; HPLC elution time (E_t) 10.2 min; IR (Nujol), ν_{max} 3320 (OH and NH), 1690 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.73 (s, 3, 18-methyl), 1.28 (t, J = 7 Hz, 3, OCH_2CH_3), 4.13 (q, J = 7 Hz, 2, OCH_2CH_3), 4.63 (s, 1, OH), 6.57–7.08 (m, 3, Ar H); MS, m/z (relative intensity) 343 (37, M$^+$), 297 (37), 269 (3.7), 254 (14), 213 (100). Anal. ($C_{21}H_{29}NO_3$) C, H, N.

4-*O*-(Carbomethoxymethyl)hexestrol (4). To a solution of hexestrol **3** (2.12 g, 7.8 mmol) and sodium (0.19 g, 8 mmol) in methanol (100 mL) was added methyl chloroacetate (1.08 g, 10 mmol). The mixture was refluxed for 3 h, evaporated, and processed as above. After evaporation of the solvent, most remaining hexestrol was removed by trituration with CH_2Cl_2. The product was purified on preparative TLC plates by two elutions with CH_2Cl_2 to give 0.44 g of **4**, mp 124–125 °C; TLC, R_f (CH_2Cl_2) 0.30; ^{1}H NMR ($CDCl_3$) δ 0.53 (t, J = 7 Hz, 6, CH_2CH_3), 1.3 (m, 4, CH_2), 2.43 (m, 2, CH), 3.77 (s, 3, OCH_3), 4.57 (s, 2, OCH_2), 5.2 (s, 1, OH), 6.57–7.17 (m, 8, Ar H); MS, m/z (relative intensity) 342 (11.4, M$^+$), 311 (14.3), 283 (22.8), 208 (85.7), 207 (100), 192 (17.1), 179 (82.8), 135 (88.6). Anal. ($C_{21}H_{26}O_4$) C, H.

4-*O*-(Carbamoylmethyl)hexestrol (5). A mixture of hexestrol **3** (1.35 g, 5 mmol), sodium bicarbonate (0.42 g, 5 mmol), and methyl chloroacetate (0.54 g, 5 mmol) in DMF (10 mL) and H_2O (2 mL) was stirred at 90 °C overnight, evaporated, and processed as described for **2**. The mixture was triturated twice with CH_2Cl_2 to remove unreacted hexestrol. A mixture of the crude ester **4** in MeOH (50 mL) and concentrated NH_4OH (1 mL) was stirred at 25 °C for 18 h. The solvent was evaporated, and the product was taken up in EtOAc, washed with water, and dried ($MgSO_4$). The product was purified on a column packed with 0–63-μm silica gel (20 g), eluting with 1:1 EtOAc/hexane (175 mL) followed by EtOAc (50 mL), to give 0.23 g of an oil that solidified upon standing, mp 108–110 °C (from CH_2Cl_2–C_6H_6); TLC, R_f (system I) 0.06, R_f (EtOAc) 0.54; IR (KBr) ν_{max} 3000–3400 (OH), 1660 (amide) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.53 (t, J = 7 Hz, 6, CH_3), 1.27 (m, 4, CH_2), 2.47 (m, 2, CH), 3.8 (s, 1, OH), 4.47 (s, 2, OCH_2), 6.33 (br s, 2, NH_2), 6.66–7.23 (m, 8, Ar H); MS, m/z (relative intensity) 327 (0.9, M$^+$), 207 (35), 192 (90), 135 (100). Anal. (HRMS) Calcd for $C_{20}H_{25}NO_3$: 327.1833. Found: 327.1829. The acetate of **5** was prepared: mp 136–137 °C; MS, m/z (relative

(24) Katzenellenbogen, J. A.; Johnson, H. J.; Myers, H. N. *Biochemistry* **1973**, *12*, 4085.

intensity) 369 (1.1, M^+), 207 (24), 192 (100), 135 (98), 107 (24). Anal. (HRMS) Calcd for $C_{22}H_{27}NO_4$: 369.1938. Found: 369.1929.

4-*O*-(2-Aminoethyl)hexestrol (6). A mixture of the amide **5** (0.2 g) in 5 mL of 1 M B_2H_6 in THF was refluxed for 16 h, at which time TLC indicated the absence of starting material. Methanol was added and evaporated to remove boric acid as methyl borate. This procedure was repeated five times, and the residue was triturated with EtOAc and dried over P_2O_5 to give product **6** (0.12 g, 60%): mp 205–207 °C; TLC, R_f (EtOAc) 0.00, R_f (MeOH) 0.14; IR (Nujol) ν_{max} 2900–3500 (OH, NH_2) cm^{-1}, absence of amide band; MS, m/z (relative intensity) 178 (12), 135 (100), 107 (41). The diacetate of **6** was prepared: mp 134–135 °C; MS, m/z (relative intensity) 397 (0.04, M^+), 354 (0.5), 177 (37), 135 (100). Anal. ($C_{24}H_{31}NO_4$) C, H, N.

4-*O*-[2-(Carbethoxyamino)ethyl]hexestrol (7). To a mixture of the amine **6** (16 mg) in THF (2 mL) and pyridine (1 mL) was added ethyl chloroformate (4.5 mg, 1 equiv) in THF (0.45 mL). After the mixture was stirred for 5 min at 25 °C, the solvent was evaporated. The residue was taken up in EtOAc and processed as described for **2**. An analytical sample was purified on HPLC (E_t = 11.2 min): mp 88–89 °C; TLC, R_f (system I) 0.20, R_f (system II) 0.66; ^1H NMR ($CDCl_3$) δ 0.52 (t, J = 7 Hz, 6, $CHCH_2CH_3$), 1.16–1.42 (m, 7, OCH_2CH_3 and CCH_2CH_3), 2.44–2.47 (m, 2, CH) 3.55–3.62 (q, J = 5 Hz, 2, OCH_2CH_2N), 4.01–4.05 (t, J = 5 Hz, 2, OCH_2CH_2N), 4.09–4.17 (q, J = 7 Hz, 2, $COOCH_2$), 6.76–6.85 (dd, J = 8 Hz, 4, Ar H), 6.99–7.07 (dd, J = 8 Hz, 4, Ar H); MS, m/z (relative intensity) 385 (2, M^+), 340 (2), 250 (90), 204 (30), 135 (100). Anal. ($C_{23}H_{31}NO_4$) C, H, N.

C-11 Labeled 2 and 7. The $^{11}COCl_2$ was collected in EtOH for a period of 10 min to yield 38–65 mCi. To this was added a solution of the amine **1** or **6** (25 μg in EtOH) containing 1% Et_3N. The reaction mixture was left at room temperature for 10 min and injected on the HPLC column, and the radioactive peak of [^{11}C]**2** or [^{11}C]**7** was collected. The elution solvent was evaporated under a stream of helium, and the product redissolved in ethanol. Radiochemical purity was verified by TLC in both solvent systems I and II. Specific activities obtained varied from 4 to 10 Ci/mmol for [^{11}C]**2** and from 1 to 2.5 Ci/mmol for [^{11}C]**7** after HPLC purification. The nondecay-corrected radiochemical yields (after HPLC purification, about 20 min after the end of bombardment) were usually 5–10% for [^{11}C]**2** and were occasionally as low as 0.5–1.0%. In the case of the [^{11}C]**7**, the yields varied between 1 and 2%.

Estrogen Receptor Binding Assay. The affinity of the estradiol and hexestrol derivatives for estrogen receptors was determined by competition studies using the Dextran-coated charcoal method.[21] Cytosol was prepared from calf uteri. Tissue samples were homogenized at 4 °C in 5 mM phosphate buffer, pH 7.4, containing 10 mM thioglycerol and 10% glycerol. After centrifugation (1 h at 105000g), the supernatant was diluted with phosphate buffer to 1–1.8 mg of protein/mL. The [^{3}H]estradiol (NEN, 130–170 Ci/mmol) was used as the radioligand at 10^{-9} M in the final incubation mixture. Test compounds were used at seven different concentrations, from 10^{-11}–10^{-5} M. Incubations were performed at 4 °C for 4 h, in triplicate. Radioactivity in charcoal-treated incubation supernatants (0.5 mL) was mixed with 10 mL of toluene-based scintillation fluid (4.0 g of PPO, 0.05 g of POPOP, 1 L of toluene) and was measured in a Searle Analytic 92 apparatus with a counting efficiency of 50%. Additional concentrations of test compounds were studied about the 50% competition point in order to obtain a graphic estimate of the concentration required for 50% displacement of the radioligand from the receptor. The RBA value of a competitor was established by using the ratio of unlabeled estradiol concentration required for 50% receptor displacement of the corresponding [^{3}H]estradiol and the competitor concentration required for the same effect, multiplied by 100.[22]

In Vivo Studies. Immature female Fisher rats (3–4 weeks old, ca. 50 g) were used to study tissue uptake of [^{11}C]**2** and [^{11}C]**7**. Solutions of 0.2–0.3 mL containing 1–10 μCi (corresponding to a mass of about 1 pmol) of these radioactive compounds were injected via the caudal vein. If designed to show the blockage of specific receptor uptake, the solution was fortified with 18 μg of nonradiolabeled estradiol. The animals were killed by cardiac puncture 10 min after injection, and tissue samples were taken and counted for radioactivity. The animals were paired in such a manner that always two of them were injected simultaneously with the same ^{11}C-labeled estrogen preparation, one without and one with carrier estradiol. The activity distribution in untreated animals and animals treated with estradiol was compared by the paired Student's t tests. A probability value of $p < 0.05$ was considered as significant.

Acknowledgment. We are grateful to the Medical Research Council of Canada for financial support and for providing one of us (G.W.) with a visiting scientist award.

Registry No. **1**, 20989-33-7; **2**, 88803-29-6; [^{11}C]**2**, 88803-30-9; **3**, 84-16-2; **4**, 88803-31-0; **5**, 88803-32-1; **6**, 88803-33-2; **7**, 88803-34-3; [^{11}C]**7**, 88803-35-4; **8**, 50-28-2; **9**, 53-16-7; **10**, 5982-51-4; [^{11}C]ethyl chloroformate, 80495-06-3.

Synthesis and Physicochemical and Neurotoxicity Studies of 1-(4-Substituted-2,5-dihydroxyphenyl)-2-aminoethane Analogues of 6-Hydroxydopamine

Alice C. Cheng and Neal Castagnoli, Jr.*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143.
Received July 11, 1983

In an attempt to evaluate the possible relationship between the neurotoxicity of 6-hydroxydopamine and the redox properties and electrophilic reactivity of the 6-hydroxydopamine–*p*-hydroquinone/*p*-quinone system, we have synthesized a series of 6-hydroxydopamine analogues in which the C_4-hydroxy group is replaced with various electron-donating and electron-withdrawing substituents. With the aid of cyclic voltammetry, the formal oxidation potentials ($E^{\circ\prime}$) for the *p*-hydroquinone/*p*-quinone redox couples and the rates of cyclization of the *p*-quinones to the corresponding *p*-iminoquinones were determined. As expected, electron-rich *p*-hydroquinones were easily oxidized to the *p*-quinones, which underwent cyclization slowly, whereas the oxidation of electron-poor *p*-hydroquinones required higher voltages and yielded *p*-quinones, which cyclized readily at pH 7.4. The neurotoxic potential of these compounds showed that in vivo destruction of noradrenergic terminals, as measured by inhibition of norepinephrine uptake by rat heart slices, occurred only with those analogues bearing electron-donating substituents. Potent neurotoxic properties were associated only with the 4-amino and 4-hydroxy derivatives, both of which form *p*-quinones, which do not cyclize readily at pH 7.4. These results support the thesis that the *p*-quinone derived from 6-hydroxydopamine may be an important species in the mediation of the neurodestruction caused by 6-hydrodopamine.

The dopamine oxidation product 1-(2,4,5-trihydroxyphenyl)-2-aminoethane [6-hydroxydopamine (6-OHDA, **1**)] is an effective sympatholytic agent[1,2] that selectively destroys noradrenergic and dopaminergic nerve terminals.[3,4]

0022-2623/84/1827-0513$01.50/0 © 1984 American Chemical Society