PREPARATION OF TRITIUM-LABELED 4-HYDROXY-α-[p-(2-(N-PYRROLIDINYL)ETHOXY)PHENYL]-α'-NITROSTILBENE (CN-928), A BIOLOGICALLY-IMPORTANT METABOLITE OF THE ANTIESTROGEN CI-628

John A. Katzenellenbogen,* Tochiro Tatee, and David W. Robertson
School of Chemical Sciences, University of Illinois
Urbana, IL 61801, U. S. A.

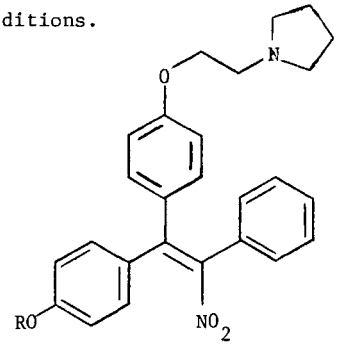
SUMMARY

3-^{3}H-4-hydroxy-α-[p-(2-(N-pyrrolidinyl)ethoxy)phenyl]-α'-nitrostilbene (^{3}H-CN-928), a tritium-labeled metabolite of the Park-Davis antiestrogen CI-628, was prepared by catalytic hydrogenolysis of the corresponding 3-iodo-CN-928 with carrier-free tritium gas. The precursor was prepared either by iodination of CN-928, or by total synthesis from 3-iodo-4-hydroxybenzoic acid. The final material was obtained with a specific activity of 22 Ci per mmole, which is sufficiently high for receptor binding studies.

Key Words: Triarylethylene antiestrogen, Friedel-Crafts acylation, Catalytic hydrogenolysis, Tritium labeling

INTRODUCTION

Studies of the molecular mechanism of antiestrogen action have been assisted by the synthesis of high specific activity tritium-labeled antiestrogens (CI-628, tamoxifen, U-23469)[1] which permits the estrogen receptor interactions of these substances to be investigated directly. We have recently described the preparation of the Park-Davis estrogen CI-628[1a] in tritium-labeled form and have investigated its interaction with the estrogen receptor in rat uterus under *in vitro* and *in vivo* conditions.



R = CH_3 CI-628

R = H CN-928

*Address reprint requests to this author.

0362-4803/81/060865-15$01.50

Received January 21, 1980
Revised March 31, 1980

While the interaction of ^{3}H-CI-628 with the uterine cytoplasmic estrogen receptor could be demonstrated in vitro, it was apparent from the in vivo studies that with time, a more polar metabolite of CI-628 was being accumulated selectively in the nuclear estrogen receptor sites. As these sites are thought to be critical mediators of estrogen and antiestrogen action, we felt that the identification of this metabolite and the study of its interaction with the estrogen receptor was of paramount importance to furthering the understanding of the molecular basis of antiestrogen action. From comparisons of chromatographic mobility in multiple solvent systems and from chemical interconversions, the CI-628 metabolite has been tentatively identified as 4-hydroxy-α-[p-(2-pyrrolidinyl)ethoxy)phenyl]-α'-nitrostilbene (CN-928), the free phenol of CI-628. This paper describes the preparation of CN-928 in high-specific activity tritium-labeled form, suitable for further studies of its metabolism and receptor interaction.

EXPERIMENTAL

Materials.

Chemicals were obtained from the following sources: Aldrich (β-bromophenetole, 4-hydroxybenzoic acid); Baker (iodine, phosphorus trichloride); Fisher (tetrahydrofuran); Linde (chlorine gas, sulfur dioxide gas); Mallinckrodt (acetic anhydride, benzyl chloride, diethyl ether, anhydrous magnesium sulfate, magnesium metal (turnings), fuming nitric acid (90%), phosphoric acid (85%), potassium iodide, zinc chloride).

Methods.

Analytical thin layer chromatography was performed using 0.25 mm silica gel glass-backed plates containing F-254 indicator (Pre-Coated TLC Plates SILICA GEL 60 F-254, Merck), and spots were visualized by 254 nm ultraviolet light. Preparative thin layer chromatography was carried out using 2 mm silica gel glass-backed plates with F-254 indicator (Pre-Coated PLC Plates SILICA GEL F-254, Merck).

Tetrahydrofuran was dried by distillation from benzophenone ketyl in a recirculating still, and diethyl ether was distilled from lithium aluminum hydride.

Melting points were determined on a Fischer-Johns apparatus and are uncorrected. Infrared spectra were determined as KBr pellets or neat films using a Beckman Model IR-12 Instrument. The data are presented in cm^{-1} and only the important diagnostic bands are reported. Proton magnetic resonance (^{1}H-NMR) spectra were taken on Varian Associates spectrometers, Model EM-390, HA-100, and HR-220. Chemical shifts are reported in ppm downfield from a tetramethylsilane internal standard (δ scale). The ^{1}H-NMR data are presented in the form: (solvent in which spectra were taken), δ value of signal (peak multiplicity, integrated number of protons, coupling constant (if any), and assignment). Mass spectra were recorded from Varian MAT CH-5 and 731 (high resolution mass spectra) spectrometers, at the ionization voltage expressed in the parentheses. Only the peaks of high relative intensity or of diagnostic importance are presented in the form: m/e (intensity relative to base peak). UV spectra were measured on a Cary-15 Instrument, using the solvent noted in the parentheses. Elemental analyses were provided by the Microanalytical Service Laboratory of the University of Illinois.

A standard procedure for product isolation was used in all reactions except where otherwise mentioned: quench with water, exhaustive extraction with a solvent, drying over an anhydrous salt (written in the parenthesis), filtration, and evaporation of solvent under reduced pressure.

Radiochemical purity was measured on plastic-backed silica gel thin layer plates (Eastman chromatogram Sheet No. 6061, without fluorescent indicator). The labeled material was spotted on top of unlabeled carrier. After development in the solvent system indicated the chromatogram was cut into ten strips, which were then placed in minivials with 5 ml of a xylene-base scintillation fluid containing 0.55% 2,5-diphenyloxazole, 0.01% p-bis[2-(5-phenyloxazolyl)] benzene, and 25% Triton X-114. Radioactivity was determined in a Nuclear Chicago Isocap 300 Liquid Scintillation Counter.

Synthesis

4-(2-Bromoethoxy)-4'-hydroxybenzophenone (3a) - To freshly prepared polyphosphoric acid (310 g) were added 85% phosphoric acid (150 g), freshly-ground zinc chloride (106 g, 778 mmol), 4-hydroxybenzoic acid (28 g, 203 mmol) and β-bromophenetole (42 g, 209 mmol). The solution was heated to 50-60 °C, phosphorus trichloride (79 g, 575 mmol) was added dropwise during 1 h, and then the reaction mixture was stirred for 20 h at 70 °C. The cooled reaction mixture was poured into 1.5 L of water and left at room temperature overnight. The solid obtained after filtration was dissolved in 400 mL of 3 N sodium hydroxide at 60 °C and filtered. The filtrate was acidified to pH 2.9 with concentrated hydrochloric acid, and then aqueous sodium bicarbonate was used to adjust the pH to 6.5. The precipitate thus obtained was dissolved in hot ethyl acetate, dried ($MgSO_4$), and recrystallized from ethyl acetate to give 48.53 g (74.4%) of product (in 3 crops). Another recrystallization from ethyl acetate gave a pure sample, mp 139-142; IR (KBr) 3240, 1632, 1608, 1258; ^{1}H-NMR (acetone-d_6) δ 3.77 (t, 2H, J = 5 Hz), 4.45 (t, 2H, J = 5 Hz), 6.8-7.9 (m, 8H, aromatic); mass spectrum (70 eV) m/e (rel intensity) 332 (42, M^+), 320 (42, M^+), 229 (21), 227 (23), 121 (100), 109 (20), 107 (21), 93 (23), 65 (29).

Anal. Calcd for $C_{15}H_{13}BrO_3$: C, 56.10; H, 4.08; Br, 24.88. Found: C, 56.69; H, 4.15; Br, 24.33.

1-(4-Acetoxyphenyl)-1-[4-(2-bromoethoxy)phenyl]-2-nitro-2-phenylethylene (6a) - Benzyl magnesium chloride was prepared from 2.0 g (82 mmol) of magnesium and 9.9 g (78 mmol) of benzyl chloride in 25 mL of dry ether. A solution of 10 g (31 mmol) of the benzophenone 3a in tetrahydrofuran:ether (1:1) was added over a 10 minute period, and the mixture was stirred under reflux for 3 h. The reaction was cooled, quenched with 70 mL of saturated ammonium chloride, acidified with dilute hydrochloric acid, and extracted with ether. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. TLC and IR analyses of the residue (13.49 g) indicated almost complete disappearance of the benzophenone.

The crude triarylethanol 4a (13.49 g) was dissolved in 60 mL of acetic anhydride and refluxed for 16 h. The solution was concentrated under reduced pressure to give 14.92 g of an oil. Dehydration and acetylation had occurred as evidenced by TLC and IR analyses.

The unpurified triarylethylene 5a (14.92 g) and 2.10 g (30 mmol) of fuming nitric acid (90%) were added to 52 mL of glacial acetic acid; the reaction was then stirred for 45 minutes at 70 °C. After cooling, the reaction was neutralized with dilute aqueous sodium bicarbonate and extracted with ether. The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give 17.58 g of an oil. Crystallization from hexane:ethyl acetate afforded yellow crystals of 6a (4.83 g, 32% overall yield from the benzophenone 3a): first crop (4.10 g) mp 117-122; second crop (0.73 g) mp 105-112; IR (KBr) 1773, 1610, 1530, 1205; ^{1}H-NMR ($CDCl_3$) 2.24 and 2.29 (each s, 3H altogether, CH_3COO-), 3.57 and 3.62 (each t, 2H altogether, J = 7 Hz, $-CH_2Br$), 4.21 and 4.28 (each t, 2H altogether, J = 7 Hz, $-OCH_2$-), 6.6-7.4 (m, 13H, aromatic); mass spectrum (70 eV) m/e (rel intensity) 483 (37, M^+), 481 (37, M^+), 441 (23), 439 (26), 437 (20), 395 (100), 393 (99), 349 (23), 286 (32), 258 (23), 257 (25), 121 (22).

Anal. Mol wt calcd for $C_{24}H_{20}BrNO_5$: 483.0505. Found: 483.0507

4-Hydroxy-α-[p-(2-(N-pyrrolidinyl)ethoxy)phenyl]-α'-nitrostilbene (CN-928, 1c) - The bromo-compound 6a (0.56 g, 1.16 mmol) and pyrrolidine (1.70g, 24 mmol) were refluxed in isopropanol (30 mL) for 5 h. Concentration of the solution in vacuo and recrystallization of the resulting residue from pyridine:water gave 0.44 g (88%) of CN-928 (1c): mp 200-203; IR (KBr) 3480, 1608, 1525, 1287, 1254, 840; ^{1}H-NMR (pyridine-d_5) 1.62 (m, 4H), 2.48 (m, 4H), 2.77 and 2.83 (each t, 2H altogether, J = 6 Hz), 4.00 and 4.09 (each t, 2H altogether, J = 6 Hz), 6.7-7.8 (m, 13H, aromatic); mass spectrum (70 eV) m/e (rel intensity) 431 (31), 430 (100, M^+), 413 (23), 287 (27), 270 (21), 252 (23), 239 (38), 194 (45), 165 (43), 152 (20), 103 (24); UV γ_{max}^{MeOH} 285 (13,200) 240 (20,000).

Anal. Mol wt calcd for $C_{26}H_{26}N_2O_4$: 430.1892. Found: 430.1890.

4-Hydroxy-α-[p-(2-(N-pyrrolidinyl)ethoxy)phenyl]-α'-nitro-3-iodo-stilbene (Iodo-CN-928, 1e) - CN-928 (205 mg, 0.477 mmol) was suspended in methanolic ammonium hydroxide (5 mL of 30% aqueous NH_4OH in 10 mL methanol). Iodine (140 mg, 0.551 mmol) in 5 mL of tetrahydrofuran was added with stirring, and the reaction was allowed to proceed at room temperature for 3 h. Saturated brine and saturated

sodium thiosulfate were added, and the mixture was extracted with ethyl acetate. Drying ($MgSO_4$) and concentration in vacuo gave 303 mg of a residue which was purified by preparative TLC (acetone:triethylamine (1:1), three developments), furnishing 111 mg (42%) of iodo-CN-928 (1e): mp 135-140 (after recrystallization from ethanol); IR (KBr) 3470, 1608, 1530, 1300, 1255; ^{1}H-NMR (CD_3OD) 1.86 (m, 4H) 2.84 (m, 4H), 3.06 (t, 2H, J = 5 Hz), 4.14 (t, 2H, J = 5 Hz), 6.5-7.4 (m, 12H, aromatic); mass spectrum (10 eV) m/e (rel intensity) 556 (5, M^+), 459 (12), 413 (13), 149 (10), 98 (14), 84 (100), 71 (16), 70 (11).

Anal. Mol wt calcd for $C_{26}H_{25}IN_2O_4$: 556.0858. Found: 556.0861

4-Hydroxy-3-iodobenzoic acid (2b) - A sodium hypochlorite solution was prepared by passing chlorine gas through a solution of 45 g (1.13 mmol) sodium hydroxide in 300 mL water until 33.1 g (466 mmol) of chlorine was absorbed. 4-Hydroxybenzoic acid (25 g, 181 mmol) was dissolved in 50 mL water containing 14.5 g (363 mmol) sodium hydroxide. Potassium iodide (41 g, 246 mmol) in 330 mL water was added, followed by 150 mL water and 500 g ice. A portion of the hypochlorite solution prepared above (165 mL, 233 mmol) was added over a 5 min period, with stirring continued for 20 min. Concentrated sulfuric acid (15 mL) was added, and sulfur dioxide gas was passed through the suspension for 30 min until the brown iodine color was dispelled. The precipitated product was collected by filtration, washed with water and recrystallized from 95% ethanol. The first two crops (8.18 g, 11.6%) gave material that analyzed for 3,5-diiodo 4-hydroxybenzoic acid. The third and fourth crops gave 34.2 g (72%) of the moniodo acid mp 169-173° (lit.[2] mp 173.5-174.5).

4-(2-Bromoethoxy)-4'-hydroxy-3'-iodobenzophenone (3b) - Freshly ground zinc chloride (9.4 g, 69 mmol), 85% phosphoric acid (15 g), 3-iodo-4-hydroxybenzoic acid (2b, 5.2 g, 19.7 mmol), and β-bromophenetole (4.5 g, 22.4 mmol) were added to freshly-prepared polyphosphoric acid (28.9 g) resulting in a pink, viscous solution. While maintaining the stirred solution at 50 °C, phosphorus trichloride (7.87 g, 57.3 mmol) was added dropwise over a 1 h period. After the addition was complete, the temperature was raised to 70 °C, and the reaction was stirred for 2.5 h. The acylation reaction was allowed to cool, 200 mL of water was

added, and the precipitated product was collected by filtration. The product was dissolved in 3 N sodium hydroxide (40 mL) at 60 °C, and the solution was filtered. The filtrate was acidified to pH 4.7 with concentrated hydrochloric acid and then the pH was adjusted to 6.5 by the slow addition of sodium carbonate. The resulting phenolic precipitate was collected by filtration and was first recrystallized from hexane:ethyl acetate and then from benzene to give the benzophenone 3b (0.93 g, 11%): mp 175-176.5; IR (KBr) 3250, 1635, 1602, 1550, 1260; ^{1}H-NMR (DMSO-d_6) δ 3.80 (t, 2H, J = 5 Hz), 4.42 (t, 2H, J = 5 Hz), 6.8-8.1 (m, 7H, aromatic), 10.73 (s, 1H, phenolic); mass spectrum (70 eV) m/e (rel intensity) 448 (M^+, 99), 446 (M^+, 100), 247 (45), 229 (52), 227 (52), 121 (36), 109 (39), 107 (42), 92 (31).

Anal. Calcd. for $C_{15}H_{12}BrIO_3$: C, 40.30; H, 2.71; Br, 17.87; I, 28.38. Found: C, 40.40; H, 2.53; Br, 17.95; I, 28.14.

1-(4-Acetoxy-3-iodophenyl)-1-[4-(2-bromoethoxy)phenyl]-2-phenylethylene (5b) - Benzyl magnesium chloride was prepared from magnesium (75 mg, 3.08 mmol) and excess benzyl chloride in 4 mL of dry ether. This Grignard reagent was added to a solution of the benzophenone 3b (300 mg, 0.67 mmol) in tetrahydrofuran (5 mL), and the reaction was refluxed for 2.5 h. After cooling, saturated ammonium chloride was added, and the mixture was extracted with ether. Drying ($MgSO_4$) and concentration in vacuo of the ether extract produced 0.51 g of the triarylethanol 4b.

The unpurified 4b (0.41 g) was dissolved in acetic anhydride (5 mL) and refluxed for 7 h. Evaporation of the solvent in vacuo followed by dry column chromatography[3] (silica gel, developed first with light petroleum ether:ethyl ether (10:1) and then light petroleum ether:ethyl ether (5:1)) gave the desired product 5b (0.17 g, 56% from the benzophenone 3b) as an oil: ^{1}H-NMR (CCl_4) δ 2.34 (s, 3H, -C<u>H</u>$_3$), 3.57 (m, 2H, -C<u>H</u>$_2$Br), 4.25 (m, 2H, -C<u>H</u>$_2$O), 6.77-7.60 (m, 13H, aromatic and olefinic); mass spectrum (70 eV) m/e (rel intensity) 564 (M^+, 2), 562 (M^+, 2), 522 (5), 520 (4), 150 (42), 108 (100), 91 (68), 43 (95).

Anal Mol wt calcd for $C_{24}H_{20}BrIO_3$: 563.9619. Found: 563.9619.

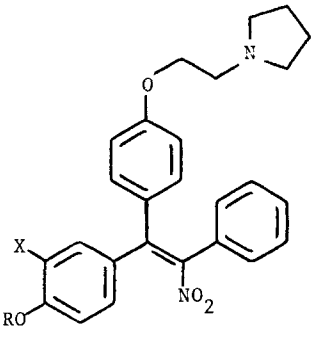
4-Hydroxy-α-[p-(2-(N-pyrrolidinyl)ethoxy)phenyl]-α'-nitro-3-iodo-stilbene (Iodo-CN-928, 1e) - The triarylethylene 5b (0.17 g, 0.30 mmol) in 0.5 mL of acetic acid was added to a solution of 90% fuming nitric acid (31 mg, 0.443 mmol) in acetic acid (0.3 mL). The reaction was stirred at 60-70 °C for 2 h, cooled, and quenched with water. The mixture was extracted with ether and the organic extract was dried ($MgSO_4$) and concentrated in vacuo to furnish 0.17 g of 6b as an oil.

The crude nitroethylene 6b (0.15 g) and pyrrolidine (170 mg, 2.4 mmol) were refluxed in isopropanol (5 mL) for 11 h. The solvent was removed under reduced pressure and the residue was purified by preparative TLC on silica gel (methanol). The iodo-CN-928 (1e; 80 mg, 54% from 5b) obtained was identical to the 1e prepared from 1c as described previously.

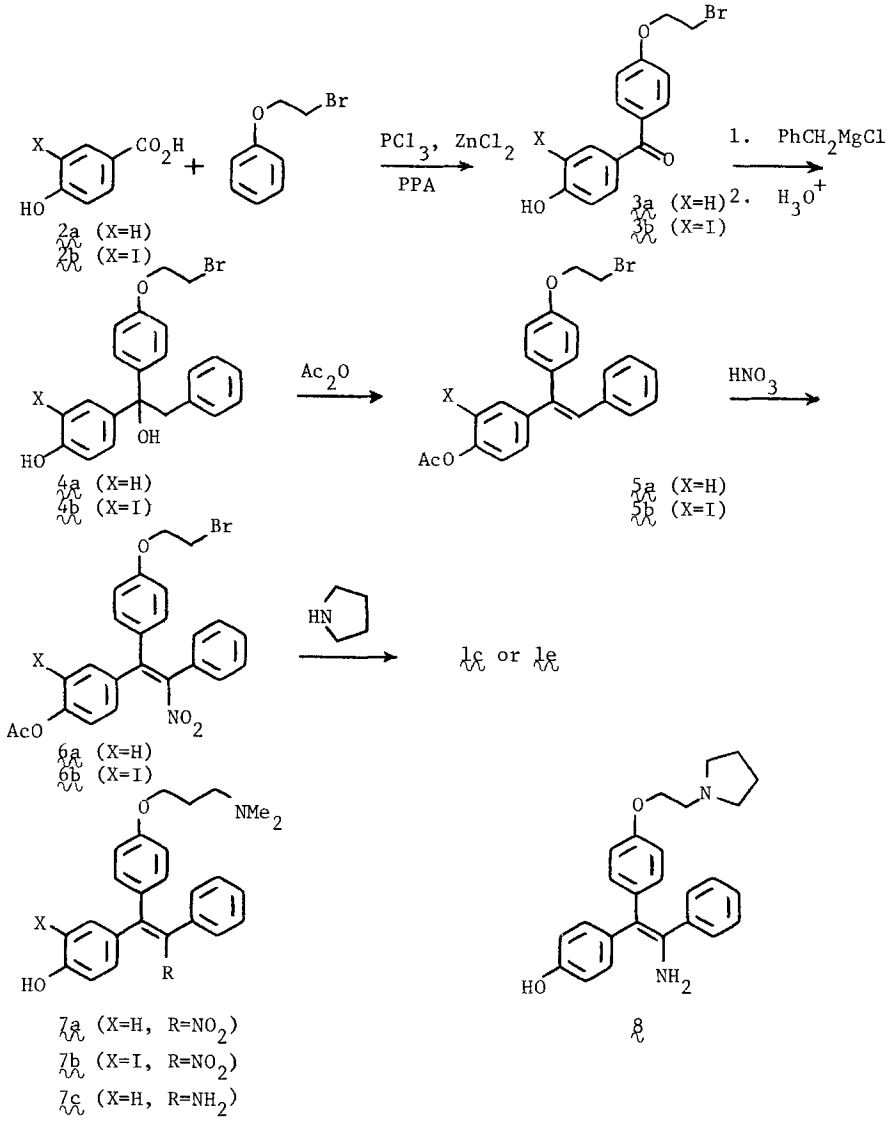
*Hydrogenolysis of iodo-CN-928 (1e). Preparation of ^{3}H-CN-928 (1d)** - Iodo-CN-928 (56 mg, 0.1 mmol) was dissolved in 2.0 mL of dimethylformamide. To this solution was added 150 mg of 5% palladium on alumina, and the reaction mixture was stirred at room temperature under 25 Ci of carrier-free tritium gas for 55 min. An uptake of 4.0 mL was noted. Labile tritium was removed, in vacuo, using benzene:ethanol (4:1) as solvent. After filtration from the catalyst, the product was taken to dryness in vacuo once more, and redissolved in 10 mL of benzene:ethanol (9:1): 3.231 Ci was produced, indicating a specific activity of 32.31 Ci/mmol before purification. 500 mCi of ^{3}H-CN-928 was shipped to us, and the radiochemical purity upon arrival in our laboratories was 60%; after storage of the 500 mCi in 3 mL of benzene:ethanol (9:1) at -25 °C for 1 month, the purity had fallen to 42%. In a trial purification 9.16 mCi of the hydrogenation product was chromatographed on a 1x20 cm column packed with deactivated silica gel (150 mL of water/kg of silica gel) using acetone:triethylamine (5:1) as the eluting solvent. Fractions of approximately 1.5 mL (32 drops/minute) were collected;

* The tritiation was done at New England Nuclear using conditions developed in our laboratory. The purification was performed in our laboratory.

the radioactivity in each fraction was assayed and fractions of similar radiochemical purity were combined. By this procedure, 3.6 mCi of ^{3}H-CN-928 of 92% radiochemical purity and 0.35 mCi of 89% radiochemical purity were obtained. The recovery of pure ^{3}H-CN-928 based upon the total radioactivity loaded on the column was 43%. A superior method for the radiochemical purification was preparative TLC (silica gel, 20x20 cm plates with a layer thickness of 0.25 mm). The ^{3}H-CN-928 in benzene:ethanol (9:1) was streaked on the plate, and the plate was developed with acetone:triethylamine (5:1). The ^{3}H-CN-928 was easily observed as a bright yellow band with an R_f of 0.30. The upper, middle, and lower zones of the band were removed separately,[4] and the ^{3}H-CN-928 was eluted from the silica gel using acetone:triethylamine (5:1). The radiochemical purity of each portion was determined, the solvent was removed under a gentle stream of nitrogen, and the yellow residue was placed in ethanol:benzene (3:1), at concentrations of $\leq$1 mCi/mL, for long-term storage at -195 °C (liquid nitrogen). In a typical purification using 73 mCi of crude ^{3}H-CN-928, the 3 fractions each had a radiochemical purity of 97%, with the combined recovery being 24 mCi (33%). A total of 148 mCi of ^{3}H-CN-928 of 96% radiochemical purity was obtained, representing 30% of the total radioactivity present in the unpurified material.



1a	R=CH_3	X=H	CI-628
1b	R=CH_3	X=^{3}H	^{3}H-CI-628
1c	R=H	X=H	CN-928
1d	R=H	X=^{3}H	^{3}H-CN-928
1e	R=H	X=I	


X
CO2H
HO
Br
O
PCl3, ZnCl2
PPA
1. PhCH2MgCl
2. H3O+
2a (X=H)
2b (X=I)
3a (X=H)
3b (X=I)
OH
4a (X=H)
4b (X=I)
Ac2O
AcO
5a (X=H)
5b (X=I)
HNO3
NO2
6a (X=H)
6b (X=I)
HN
1c or 1e
NMe2
R
7a (X=H, R=NO2)
7b (X=I, R=NO2)
7c (X=H, R=NH2)
N
NH2
8

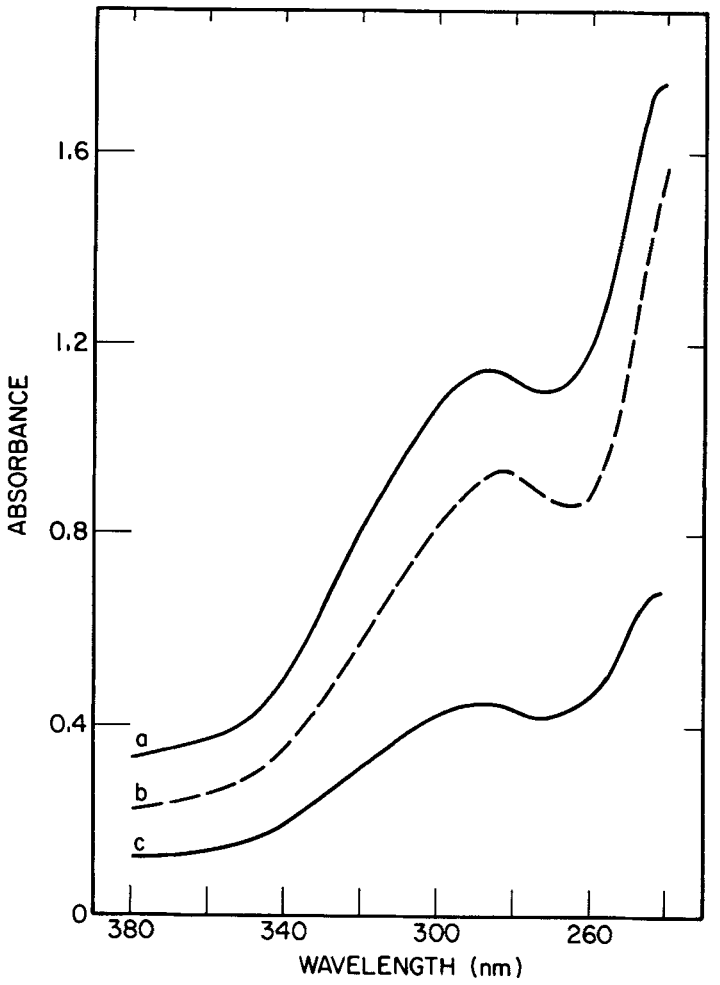


Figure 1. Ultraviolet spectrum and specific activity determination of ^{3}H-CN-928. Unlabeled CN-928 was dissolved in ethanol at 9.5×10^{-5} M (curve a) and 3.4×10^{-5} M (curve c), and the UV spectra were run on a Varian 635D spectrophotometer. A sample of ^{3}H-CN-928 of measured radioactivity was also scanned in ethanol (curve b). Comparison of the absorbance at 286 nm indicated a specific activity of 22 Ci/mmol for ^{3}H-CN-928.

Determination of the specific activity of ^{3}H-CN-928 (1d) - CN-928 has an ultraviolet absorbance pattern with maxima at 240 (ε= 20,000) and 286 nm (ε= 13,200); the ultraviolet spectrum of ^{3}H-CN-928 is very similar to that of CN-928 (Fig. 1). The specific activity was found to be 22 Ci/mmol (51 μCi/μg).

RESULTS AND DISCUSSION

We first attempted to prepare ^{3}H-CN-928 (1d) simply by demethylation of ^{3}H-CI-628 since we had a supply of ^{3}H-CI-628 (1b). However, treatment of ^{3}H-CI-628 with 48% hydrobromic acid in refluxing acetic acid,[5] with boron tribromide in methylene chloride,[6] with methionine in methanesulfonic acid,[7] or with lithium diphenylphosphide in THF[8] gave either multiple products or polar material, as analyzed by TLC.

We were able to prepare ^{3}H-CN-928 by the reduction of an iodinated precursor 1e with carrier-free tritium gas. The precursor was prepared by two routes: the first paralleled the synthesis of 9411X27 (7a) as described by DeWald.[9] β-Bromophenetole was acylated with 4-hydroxybenzoic acid in 74% yield using phosphorous trichloride-zinc chloride in polyphosphoric acid. Protection of the phenolic hydroxyl group is not necessary. The next three steps were done without purification of the intermediates in 32% overall yield: addition of benzyl Grignard, acetylation with concomitant dehydration of the tertiary benzylic alcohol, and nitration at the vinylic position. Finally, reaction with pyrrolidine gave CN-928 (1c) in 88% yield. Monoiodo CN-928 (1e) was prepared in 42% yield by treatment of CN-928 with 1.15 equiv of iodine in methanolic ammonium hydroxide. Attempts to prepare the diiodo derivative using a larger excess of iodine gave several products.

The second approach to the iodinated precursor began with 3-iodo-4-hydroxybenzoic acid (2b), prepared in 72% yield by iodination of 4-hydroxybenzoic acid. The iodo acid acylated bromophenetole only in low yield (11%), however. Nevertheless, this material (3b) could be carried through the subsequent steps to give 1c in 30% yield overall from 3b.

In model studies of the hydrogenolysis reaction, exposure of iodinated 9411X27 (7b), a CN-928 analog, to hydrogen over a palladium on alumina catalyst in DMF at room temperature cleanly effected hydrogenolysis to 9411X27 (7a) within 30 min. However, continued exposure to hydrogen for 8 hr also reduced the nitro group, giving the enamine 7c. This overreduction product (7c) has chromatographic properties similar to 9411X27 (7a); however, it UV spectrum shows a λ_{max}^{EtOH} at 276 nm (vs 283 nm for CI-680), and it displays a molecular ion at m/e 388 (vs 418 for 9411X27).

Incorporation of tritium was done at New England Nuclear by exposure of iodo-CN-928 to 25 Ci of carrier-free tritium gas over a palladium on alumina catalyst in DMF for 55 min. Approximately 500 mCi of the tritiated product was returned to us as a solution in 3 mL of benzene:ethanol (9:1). Examination of the crude product indicated 60% of the radioactivity cochromatographed with CN-928; however, after storage at -25 °C for one month prior to purification this diminished to 42%.

Although considerable purification of the ^{3}H-CN-928 could be effected by column chromatography on deactivated silica gel, preparative TLC on silica gel proved to be a superior method. Up to 73 mCi could be applied to each 20x20 cm plate (0.25 mm layer). After development of the plate, the yellow band containing the product was removed and the ^{3}H-CN-928 eluted from the silica gel.

The combined yield of purified ^{3}H-CN-928 was 148 mCi, representing 30% of the material in the crude hydrogenation product (or 70% of the material that cochromatographed as CN-928). Chromatographic analysis showed this material to

have a radiochemical purity of 96%. It was stored at ≤1 mCi per mL in 3:1 ethanol benzene at liquid nitrogen temperatures.

The identity of ^{3}H-CN-928 as prepared above was further ascertained by ultraviolet spectroscopy (Figure 1). The absence of a peak or shoulder at approximately 276 nm indicates that there is little or no contamination from the enamine 8. Furthermore, from absorbance measurements at 286 nm, it is possible to calculate the specific activity of this material as 22 Ci per mmole (51 μCi per μg). Hence this material is of sufficiently high specific activity to enable detailed studies of its estrogen receptor binding properties to be undertaken. Such studies should give insight into the molecular bases of antiestrogen antagonist action.

ACKNOWLEDGMENT

This research was supported by grants from the National Institutes of Health (AM 15556 and CA 18119). J.A.K. is a Camille and Henry Dreyfus Teacher-Scholar and D.W.R. was supported by a fellowship from the University of Illinois. We are grateful to Drs. Jerry Reel and Horace DeWald (Warner Lambert-Parke Davis) for gifts of the antiestrogens CI-628, 9411X27, and CN-928 and for procedural details for the synthesis of CN-928.

REFERENCES

1. (a) CI-628: Katzenellenbogen B. S., Katzenellenbogen J. A., Ferguson E. R., and Krauthammer N. - J. Biol. Chem. 253: 697 (1978); E. R. DeSombre and C. R. Lyttle, in "Hormones, Receptors, and Breast Cancer," Progress in Cancer Research and Therapy, vol 10. W. L. McGuire, Ed., Raven Press: New York, NY p 181 (1978).

(b) U-23469: Tatee T., Carlson K. E., Katzenellenbogen J. A., Robertson, D. W., and Katzenellenbogen B. S. - J. Med Chem. 22: 1509 (1979).

(c) Tamoxifen: Capony F. and Rochefort H. - Molec. Cell. Endocrinol. 11: 181 (1978); Jordan V. C., Dix C. J., Naylor K. E., Prestwich G., and Rowsby L. - J. Toxicol. Environ. Health 4: 363 (1978).

2. Weast R. C., Ed., "Handbook of Chemistry and Physics," 52nd ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1971, p C-187.
3. Loev B. and Goodman M. M. in "Progress in Separation and Purification," Vol. 3, Perry E. S. and Van Oss C. J., eds., Wiley-Interscience, New York, 1970, pp 73-95.
4. A TLC zone collector has been described: Platt S.G. - Anal. Biochem. 91: 357 (1978).
5. Long L. L. Jr. and Burger A.- J. Org. Chem. 6: 852 (1941).
6. McOmie J. F. W., Watts M. L. and West D. E. - Tetrahedron 24: 2289 (1968).
7. Irie H., Fujii N., Ogawa H., Yajima H., Fujino M., and Shinagawa S.- J. Chem. Soc. Chem. Comm. 922 (1976)
8. Mann F. G. and Pragnell M. J. - J. Chem. Soc. 4120 (1965).
9. Dewald H. A.- Parke-Davis Co., personal communication.