SYNTHESIS OF RADIOIODINATED ANALOGS OF 2-PHENYLPYRAZOLO[4,3-c]-QUINOLIN-3(5H)-ONE BY A MODIFIED TRIAZENE METHOD

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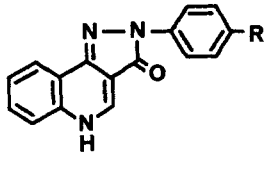
SUMMARY

No-carrier-added 2-(4-[^{131}I]iodophenyl)- and 8-[^{131}I]iodo-2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one were prepared in isolated radiochemical yields of 11.6 and 5.4%, respectively, by acid decomposition of aryl triazenes in acetonitrile. A modified triazene method involving solid-phase decomposition reactions increased the isolated radiochemical yields to 15-35%.

Key Words: Radioiodination, Iodine-131, Triazene method, Solid-phase reaction

INTRODUCTION

Recently it has been shown that 2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one (1) is a novel benzodiazepine antagonist whereas its chloro derivative (2) exhibits potent anxiolytic activity without apparent sedative liability (1,2). This unique pharmacological property prompted us to develop synthesis of radiolabeled analogs of (1) labeled with gamma and/or positron emitting nuclides. With these compounds we plan to determine their tissue distribution properties and then correlate them with structure and pharmacological activity. As a part of such a program, we initially attempted to introduce ^{131}I into the para-position of



(1) R = H
(2) R = Cl

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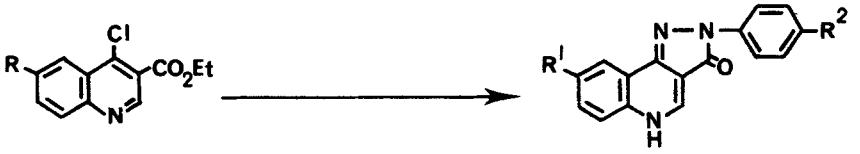
the phenyl ring and the C_8-position of (1), which might retain biological activities associated with the pyrazoloquinoline structure. A variety of efficient procedures for the preparation of radioiodinated aromatic compounds with high specific activity have been recently reported (3-5). In the present work we selected the Wallach triazene reaction which has previously been utilized for radiofluorination (6-9). This decomposition reaction can be performed rapidly and the high yields have been obtained when used for radioiodination (10,11).

RESULTS AND DISCUSSION

The triazene intermediates (9) and (10) were prepared by the following sequence of reaction (Scheme 1). The nitro compounds (5) and (6) were synthesized, according to a method described by Yokoyama et al (2), by the reactions of ethyl 4-chloroquinolin-3-carboxylate (3) and ethyl 4-chloro-6-nitroquinolin-3-carboxylate (4) with p-nitrophenylhydrazine and phenylhydrazine, respectively. The amino intermediates (7) and (8) obtained by reduction with sodium sulfide were then diazotized using sodium nitrite and methanesulfonic acid and the diazonium salts on coupling with excess of piperidine and pyrrolidine gave the expected triazene (9) and (10) in 47 and 89% yields, respectively, after column chromatography. These triazenes are quite stable substances and can be stored at room temperature.

The decomposition of the triazenes using non-radioactive sodium iodide to prepare 2-p-iodophenyl (11) and 8-iodo (12) derivatives was studied under a variety of experimental conditions. The combination of acetonitrile as solvent and methanesulfonic acid as acid led to the best yield of the iodinated compound, when three equivalents of the acid and four equivalents of sodium iodide were used. Thus treatment of (9) in refluxing acetonitrile for 50 min under these conditions gave (11) in 64% yield with the protonated by-product (1)(34%). The triazene (10) under the same conditions was more effectively converted into (12) (85%). The use of the further increase of the acid or equimolar amounts of sodium iodide to the triazenes resulted in decreased formation of the desired compounds,



(3) R = H
(4) R = NO_2

(5) R^1 = H R^2 = NO_2
(6) R^1 = NO_2 R^2 = H
(7) R^1 = H R^2 = NH_2
(8) R^1 = NH_2 R^2 = H
(9) R^1 = H R^2 = N=N-N
(10) R^1 = N=N-N R^2 = H
(11) R^1 = H R^2 = I
(12) R^1 = I R^2 = H
(13) R^1 = H R^2 = ^{131}I
(14) R^1 = ^{131}I R^2 = H

Scheme 1

as shown by HPLC analysis of the reaction mixtures with 1 mg of the triazenes in 1-2 ml of acetonitrile. The complete formation of the diazonium salts from the triazenes at room temperature was found to occur at a molar ratio of three for the acid to the triazenes.

Based on the preliminary information about the reaction conditions described above, the radioiodinations were attempted using no-carrier-added $Na^{131}I$. After we had carried out the preliminary labeling experiments, preparative labeling with 1-2 mCi of $Na^{131}I$ was performed by heating the triazenes (1 mg) with three equivalents of methanesulfonic acid in acetonitrile (1-2 ml) under reflux. Reaction times of 60 min for the complete decomposition of the triazenes were required. After separation by HPLC using a normal phase column and reverse phase column, the required (13) and (14) with no-impurities detectable by UV absorbance or radioactivity were typically isolated in 11.6 and 5.4% yield,

respectively. The purification of the products took about 1-1.5 hr.

The reasons for the low yields are not clear, but the amount of $Na^{131}I$ in the no-carrier-added synthesis are very small with the respect to the triazene intermediates. This may lead to the predominant formation of the side products from reaction with solvent molecule and to some extent decomposition of the diazonium salt itself, as occurs in the case of radiofluorination (12-15). In fact, HPLC analysis of the reaction mixture indicated many by-products and the major non-radioactive product was the protonated compound (1), as expected. In spite of the fact that aryl triazene is an attractive precursor for radioiodination of simple aromatic compounds, the present triazene method to prepare (13) and (14) at high specific activity is unsatisfactory. Recently, the use of chlorinated solvents such as carbon tetrachloride and trichloroacetonitrile has been suggested as a good solvent for radiofluorination and radiobromination using triazenes (12,16,17), as they will suppress the formation of the protonated product. These solvents, however, suffered from the formation of the chlorine substituent product as indicated by thin layer chromatographic analysis using reverse-phase plates.

A popular method of radioiodination involves simply heating labeled sodium iodide with a melt of iodinated or brominated compound to be labeled (18,19). Recently solid-phase exchange radioiodination of aryl iodides in the presence of ammonium sulfate has been developed by Mangner et al (20). Efforts to improve the radiochemical yield and decrease reaction time led to the examination of a modified procedure based on the triazene method, which might prevent the formation of the side products derived from solvent molecule. This modification consisted of adding the diazonium salts prepared in acetonitrile using 1 mg of the triazenes to a reaction vessel containing the radioactivity, heating to allow escape of the solvent (300 µl) by distillation at 130°C in 2-3 min, and additional heating the reaction vessel at the same temperature for 30 min in an open system. Initially we used the acid-to-triazene ratio of 2:1, but it was found that a single equivalent of methanesulfonic acid was sufficient for the decomposition of the triazenes. During the labeling reaction the majority of

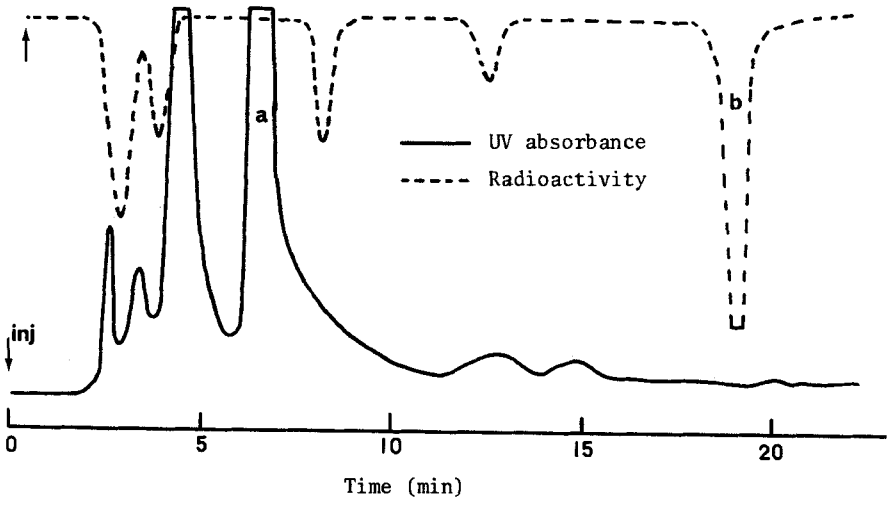


Fig. 1. A typical chromatogram obtained by HPLC using a reverse phase column from synthesis of 2-(4-[^{131}I]iodophenyl)-pyrazolo[4,3-c]quinolin-3(5H)-one via the triazene (9)(conditions described in the text).
(a) 2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one
(b) 2-(4-[^{131}I]iodophenyl)-pyrazolo[4,3-c]quinolin-3(5H)-one

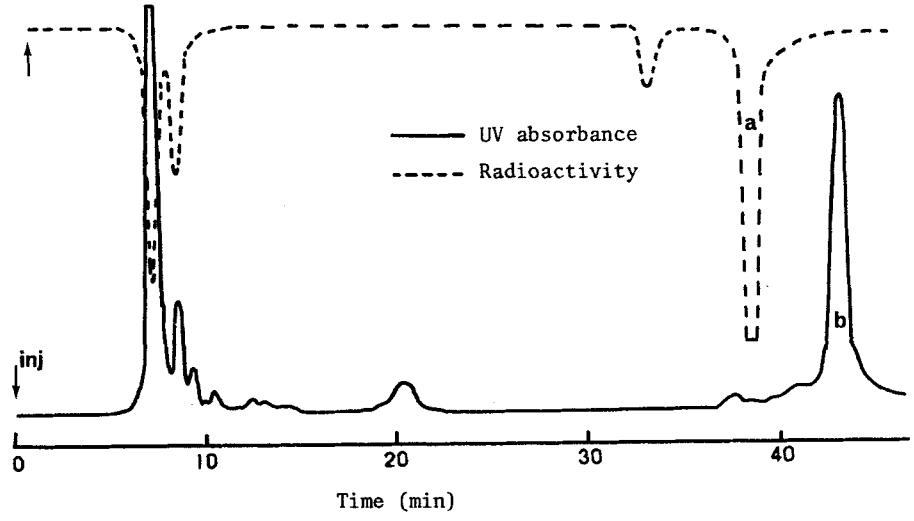


Fig. 2. A typical chromatogram obtained by HPLC using a normal phase column from synthesis of 8-[^{131}I]iodo-2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one via the triazene (10)(conditions described in the text).
(a) 8-[^{131}I]iodo-2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one
(b) 2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one

label incorporation occurred in the first 15 min, followed by a slow incorporation which was essentially finished by 30 min. The reaction mixture turned dark brown and apparently a solid-phase was maintained during this heating. This procedure increased the isolated radiochemical yield of (13) to 30-35%. Similarly with the triazene (10) the yield of (14) was raised to 15-25%. Typical chromatograms of the first separation step are shown in Fig. 1 and 2 for the decomposition of the triazenes to form (13) and (14), showing almost the same chromatographic patterns as those in solution procedure.

The ^{131}I-iodide was obtained in aqueous dilute Na_2CO_3 solution. As the labeling reaction must be performed under anhydrous conditions, it was necessary to evaporate the water before the labeling as the procedure in solution. In this step most of the radioactivity was left in a reaction vessel, but 5-10% losses of the original amount of radioactivity were observed on initial heating to remove the solvent from the reaction vessel. During the heating needed for the reaction to occur additional losses of 20-25% were found. As a result, about 65-75% of the original activity was left in the reaction mixture after completion of the reaction. However, in the modified procedure, the desired radioiodination was much more effective than that in solution. HPLC using the same chromatographic system as that in solution allowed the isolation of (13) and (14) with radiochemical purity in excess of 99% and free of UV-absorbing contaminants. As the iodinated pyrazoloquinolines have strong UV absorbances at the region of 260-390 nm, as little as 0.4-0.6 μg of product can be quantified by UV spectroscopy. Using external calibration, the specific activity of the labeled compounds was estimated to be at least more than 200 Ci/mmol. No further improvement in the isolated radiochemical yield was achieved when a sulfonic acid cation resin (Bio Rad AG 50W-X12, H^+ form)(21) was used as the acid or a decreased volume of acetonitrile used for the generation of the diazonium salts was used, even if the reaction mixture was sealed under the same conditions.

The modified procedure described here, based on the triazene method, may be also applicable to the high specific activity synthesis of other aromatic radiohalide compounds where difficulties occur in obtaining satisfactory yields by diazonium salt decomposition in an organic solvent. Although 25-35% of the

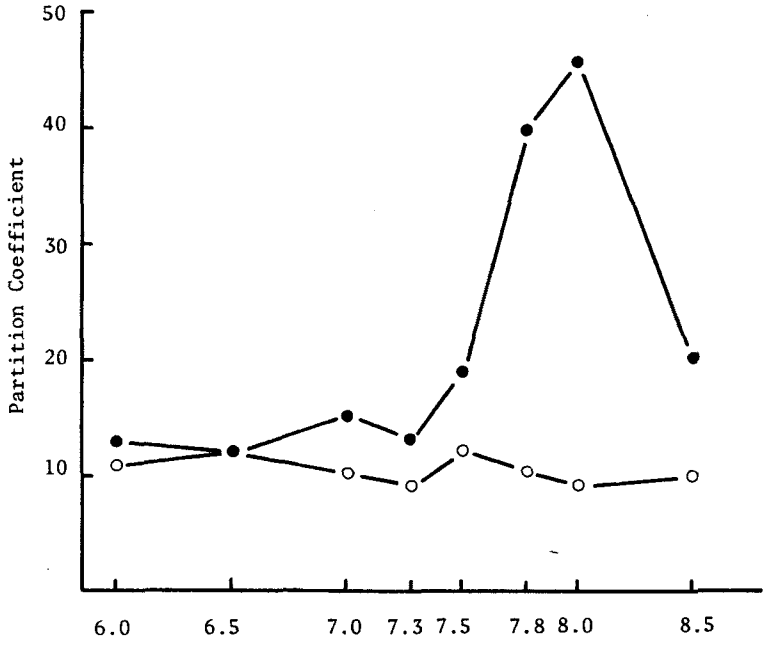


Fig. 3. Partition coefficients of radioiodinated compounds. average of four experiments: (●—●) 2-(4-[^{131}I]iodophenyl (13); (○—○) 8-[^{131}I]iodo (14)

radioiodine is lost during heating, this is not major obstacle to the application of this technique.

The partition coefficients for the radioiodinated compounds were determined at pHs from 6.0 to 8.5 at 37°C, showing high lipid-solubility of these two compounds (Fig. 3). The partition coefficient for (14) was independent on pH while that for (13) increased in the region of pH 7.3-8.0 and decreased again at pH 8.5. The biological evaluation of these compounds is currently under investigation.

Experimental

General

Proton magnetic resonance (^{1}H-NMR) spectra were obtained for solutions in deuterated dimethyl sulfoxide-d_6 with a JNM PS-100 or a JEOL FX-100 spectrometer with Me_4Si as internal reference and infrared (IR) spectra were taken on a JASCO

IRA-1 spectrophotometer. Electron-ionization (EI) and field desorption (FD) mass spectra were determined on a JEOL D-300 or DX-300 mass spectrometer and ultra-violet (UV) spectra were obtained on a Hitachi 220A spectrophotometer. Column chromatography was carried out on silica gel (silica gel 60, 70-230 mesh, Merck). Solvents were removed under reduced pressure on a rotary evaporator. Elemental analyses were performed by the staff of the microanalytical section of Kyushu University. Thin layer chromatography (TLC) using silica gel 60-F 254 (0.5 mm, Merck) and/or reverse-phase plates ($KC_{18}F$, Whatman) was used to monitor the reactions and to ascertain the purity of reaction products. Thin layer radiochromatograms were analyzed for ^{131}I using a Aloka 101 radiochromatogram scanner on the same plates. The ^{131}I used in this study was no-carrier-added solution of $Na^{131}I$ (200-500 mCi/ml) in reductant free aqueous Na_2CO_3 obtained from CIS, France. The commercial solution was diluted with methanol and the water was removed by azetropic with dry benzene prior to use. High pressure liquid chromatography (HPLC)(Waters) was carried out using a reverse phase column (25 cm Partisil M9 10/25, ODS-2, Whatman) and a normal phase column (50 cm Partisil M9 10/25, Whatman). A 2 ml injection loop was utilized. Column-effluent absorbance was monitored at 254 nm UV detector and effluent radioactivity was determined on either a Berthold LB 503 flow detector or by collection and analysis of the individual samples. Radioactivity was quantified with a Capintec Model CRC-5 radioisotope calibrator. Chemical and radiochemical purity of radioiodinated compounds was determined by TLC and HPLC; the identity was supported by HPLC co-injection studies. The specific activity was determined by UV spectroscopy and a radioisotope calibrator.

2-(4-Nitrophenyl)-pyrazolo[4,3-c]quinolin-3(5H)-one (5)

A mixture of ethyl 4-chloroquinolin-3-carboxylate (3)(22)(1 g) and p-nitrophenylhydrazine (1.95 g) in ethanol (30 ml) was refluxed for 2 hr, cooled to room temperature. The precipitate product was collected by filtration and dried

in vacuo. Recrystallization from ethanol gave (5)(923 mg, 71%) as orange needles, mp. >290°C. IR(nujol) 1650 cm^{-1}; MS m/e 306(M^+); ^{1}H-NMR δ 7.5-7.7(3H, m), 8.2-8.27(1H, m), 8.3-8.56(4H, m), 8.81(1H, s, H-4), 13.01(1H, broad, NH). Anal. Calcd for $C_{16}H_{10}N_4O_3$: C, 62.74; H, 3.29; N, 18.29. Found: C, 62.40; H, 3.22; N, 18.10.

2-(4-Aminophenyl)-pyrazolo[4,3-c]quinolin-3(5H)-one (7)

A mixture of (5)(200 mg) and Na_2S $9H_2O$ (627 mg) in ethanol (20 ml) was refluxed for 6 hr. The solvent was removed and water was added. The aqueous suspension was then neutralized with 2 N HCl. The solid material was collected by filtration, washed with water, and dried in vacuo. Chromatography [$CHCl_3$-MeOH (20:1)] gave (7)(131 mg, 73%) as light green needles, mp. >290°C, after recrystallization from 2-propanol. IR(nujol) 3210, 3300, 3350, 3450 cm^{-1}; MS m/e 276(M^+); ^{1}H-NMR δ 5.4(2H, broad s, NH_2), 6.59-6.7(2H, m), 7.49-7.8(5H, m), 8.14-8.22(1H, m), 8.49(1H, s, H-4), 12.7(1H, broad, NH). Anal. Calcd for $C_{16}H_{12}N_4O$: C, 69.55; H, 4.38; N, 20.28. Found: C, 69.35; H, 4.43; N, 20.19.

2-[4-[(2-Piperidin-1-yl)-1,2-diazaethylen-1-yl]phenyl]-pyrazolo[4,3-c]quinolin-3(5H)-one (9)

The amine (7)(484 mg) was mixed with water (24 ml) and cooled in an ice bath, and a solution of sodium nitrite (121 mg) in water (12 ml) was added with stirring at 0°C. After 5 min, a solution of methanesulfonic acid (841 mg) in water (12 ml) was added dropwise over several minutes, the temperature being kept near 0°C. After 15 min, a solution of piperidine (1.19 g) in water (12 ml) was added slowly and stirring was continued for a further 15 min. A pale yellow precipitate was collected by filtration, washed with water, and dried in vacuo. The crude triazene was chromatographed [$CHCl_3$-MeOH (39:1)] to give (9)(307 mg, 47%) as yellow needles, mp. 230-240°C(decomp.), after recrystallization from $CHCl_3$-ether. IR(nujol) 1620 cm^{-1}; ^{1}H-NMR δ 1.65(6H, broad s, protons of piperidine ring), 3.74(4H, broad s, protons of piperidine ring), 7.38-7.48(2H, m), 7.54-7.76(3H, m), 8.16-8.35(3H, m), 8.73(1H, s, H-4), 12.8(1H, broad, NH).

Anal. Calcd for $C_{21}H_{20}N_6O$: C, 67.72; H, 5.41; N, 22.57. Found: C, 67.40; H, 5.43; N, 22.43.

2-(4-Iodophenyl)-pyrazolo[4,3-c]quinolin-3(5H)-one (11)

To a suspension of the triazene (9)(100 mg) and dry sodium iodide (162 mg) in dry acetonitrile (10 ml) was added slowly methanesulfonic acid (77 mg). The mixture was refluxed with stirring for 50 min until a negative spot test was obtained with alkaline β-naphthol. After removal of the solvent, water was added and the aqueous suspension was neutralized with 1 N NaOH under cooling. The precipitate was collected by filtration, dried in vacuo, and subjected to chromatography. Elution with ethyl acetate gave (11)(67 mg, 64%) as needles, mp. >290°C, after recrystallization from methanol. IR(nujol) 1620 cm^{-1}; MS m/e 387(M^+); ^{1}H-NMR δ 7.4-7.8(5H, m), 8.02-8.27(3H, m), 8.75(1H, s, H-4), 12.5(1H, broad, NH). Anal. Calcd for $C_{16}H_{10}IN_3O$: C, 49.64; H, 2.60; N, 10.85. Found: C, 49.41; H, 2.60; N, 10.63. Further elution with the same solvent gave (1)(24 mg, 34%) as yellow needles, mp. >290°C, after recrystallization from ethanol, which was in all respects identical with an authentic sample (2).

Ethyl 4-chloro-6-nitroquinolin-3-carboxylate (4)

A mixture of phosphorus oxychloride (6 g) and ethyl 4-hydroxy-6-nitroquinolin-3-carboxylate (1 g)(23) was refluxed for 60 min, then allowed to cool somewhat and poured into ice water to which had been added conc. aqueous NH_3 (20 ml). The solid formed was collected by filtration, washed thoroughly with water, and dried in vacuo. The product was purified by chromatography ($CHCl_3$) to give (4)(0.54 g, 50%) as yellow needles, mp. 144-146.5°C, after recrystallization from ethanol. IR(nujo) 1740 cm^{-1}; ^{1}H-NMR δ 1.42(3H, t, J=7 Hz, CH_3), 4.43(2H, q, J=7 Hz, CH_2), 8.35(1H, d, J=9 Hz, H-8), 8.62(1H, dd, J=9, 2.5 Hz, H-7), 9.14(1H, d, J=2.5 Hz, H-5), 9.34(1H, s, H-2). Anal. Calcd for $C_{12}H_9ClN_2O_4$: C, 51.35; H, 3.23; N, 9.98. Found: C, 51.29; H, 3.32; N, 9.78.

8-Nitro-2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one (6)

A mixture of (4)(259 mg) and phenylhydrazine (200 mg) in ethanol (11 ml) was refluxed for 6 hr. After cooling in an ice bath, the solid material was collected by filtration, dissolved in 1 N NaOH, and filtered to remove a small

amount of insoluble material. The aqueous alkaline solution was neutralized with 2 N HCl. The precipitate formed was again collected by filtration, washed with water, and dried in vacuo. Recrystallization from ethanol gave (6)(196 mg, 69%) as a red solid, mp. >300°C, which was used in the next step without further purification. IR(nujol) 1625 cm^{-1}; MS m/e 306(M^+); ^{1}H-NMR δ 7.15-7.55(3H, m), 7.77(1H, d, J=10 Hz, H-6), 8.10-8.25(2H, m), 8.35(1H, dd, J=10, 2 Hz, H-7), 8.72(1H, s, H-4), 8.80(1H, d, J=2 Hz, H-9).

8-Amino-2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one (8)

A mixture of (6)(99 mg) and Na_2S $9H_2O$ (286 mg) in ethanol (6 ml) was refluxed for 30 min. After work-up as described for the preparation of (7), the residue was chromatographed [$CHCl_3$-MeOH (15:1)] to give (8)(66 mg, 73%) as light yellow needles, mp. >300°C, after recrystallization from methanol. IR(nujol) 1625 cm^{-1}; MS m/e 276(M^+); ^{1}H-NMR δ 5.71(2H, s, NH_2), 6.97(1H, dd, J=8.8, 2.4 Hz, H-7), 7.06-7.51(3H, m), 7.31(1H, d, J=2.4 Hz, H-9), 7.45(1H, d, J=8.8 Hz, H-6), 8.16-8.26(2H, m), 8.46(1H, s, H-4), 12.3(1H, broad, NH). Anal. Calcd for $C_{16}H_{12}N_4O$: C, 69.55; H, 4.38; N, 20.28. Found: C, 69.17; H, 4.50; N, 20.05.

8-[(2-Pyrrolidin-1-yl)-1,2-diazaethylen-1-yl]-2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one (10)

The amine (8)(406 mg) was suspended by vigorous stirring in cold water (40 ml). To this was added an ice-cold solution of sodium nitrite (125 mg) in water (4 ml) and the mixture maintained at 5°C while a solution of methanesulfonic acid (723 mg) in water (4 ml) was added dropwise. After the addition had been completed, the mixture was stirred for 30 min and a cold solution of pyrrolidine (839 mg) in water (4 ml) was added slowly. The mixture was stirred for another 1 hr and 2 N HCl was then added until pH 8.0 was attained. The solid material was collected by filtration, washed with water, and dried in vacuo. Recrystallization from ethanol gave (10)(469 mg, 89%) as yellow needles, mp. 200-230°C(decomp.). IR(nujol) 1640 cm^{-1}; MS(FD, MeOH) m/e 358(M^+); ^{1}H-NMR δ 2.00(4H, m, protons of pyrrolidine ring), 3.63-3.95(4H, m, protons of pyrrolidine ring), 7.09-7.25(1H, m), 7.35-7.62(2H, m), 7.67(2H, broad d), 8.06(1H, d, J=2.5 Hz, H-9), 8.18-8.27(2H, m), 8.65(1H, s, H-4), 12.3(1H, broad, NH). Anal. Calcd

for $C_{20}H_{18}N_6O$: C, 67.03; H, 5.06; N, 23.45. Found: C, 66.88; H, 5.11; N, 23.40.

8-Iodo-2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one (12)

A mixture of the triazene (10)(100 mg), dry sodium iodide (166 mg), and methanesulfonic acid (80 mg) in dry acetonitrile (20 ml) was refluxed with constant stirring. After 45 min, a negative spot test was obtained with alkaline β-naphthol. The solvent was removed and water was added. The aqueous suspension was then neutralized by addition of 1 N NaOH. The solid material was collected by filtration, washed with water, and dried in vacuo. Chromatography [$CHCl_3$-MeOH (30:1)] gave (12)(92 mg, 85%) as yellow needles, mp. >300°C, after recrystallization from methanol. IR(nujol) 1640 cm^{-1}; MS(FD, MeOH) m/e 387(M^+); ^{1}H-NMR δ 7.1-7.52(3H, m), 7.50(1H, d, J=9 Hz, H-6), 7.97(1H, dd, J=9, 2 Hz, H-7), 8.16-8.26(2H, m), 8.50(1H, d, J=2 Hz, H-9), 8.75(1H, s, H-4), 12.5(1H, broad, NH). Anal. Calcd for $C_{16}H_{10}IN_3O$: C, 49.64; H, 2.60; N, 10.85. Found: C, 49.72; H, 2.60; N, 10.68. TLC analysis of the reaction mixture showed the presence of (1), but isolation in a pure form was unsuccessful.

2-(4-[^{131}I]Iodophenyl)-pyrazolo[4,3-c]quinolin-3(5H)-one (13)

Method A. In a typical experiment, the triazene (9)(1 mg) was added to a reaction vessel containing dry Na^{131}I (1.89 mCi). A solution of dry acetonitrile (2 ml) containing three equivalents of methanesulfonic acid was added to the mixture. The mixture was stirred magnetically and refluxed for 60 min. The course of the reaction was monitored by TLC using a reverse phase plates [MeOH-H_2O (8:2)](R_f for 13 = 0.20). The reaction mixture was then neutralized with aqueous dilute NaOH and extracted with $CHCl_3$-MeOH (15:2). The combined extracts (1.15 mCi) were washed with water and evaporated to dryness. The residue was dissolved in MeOH-H_2O (8:2) and subjected to HPLC using a reverse phase column which had been eluted with MeOH-H_2O (8:2) at a flow rate of 4 ml/min. The fraction containing the radioiodinated compound (eluted after 19 min)(285 μCi) was collected and the eluant was removed. The second purification by HPLC performed on a normal phase column [$CHCl_3$-MeOH (97:3), 4 ml/min] gave 221 μCi (11.6%) of (13)(eluted after 35 min) with no-impurities detectable by UV absorbance or radioactivity. The synthesis time required was

about 2.5-3 hr.

Method B. Three hundreds µl of a CH_3CN solution (3 ml) containing methanesulfonic acid (2.6 mg) were added to a vial containing the triazene (9) (1 mg). The yellow suspension was subjected to an ultrasonic treatment for 30 sec. The resulting red suspension was added directly to a test tube containing dry $Na^{131}I$ (600 µCi-1 mCi). The reaction mixture was allowed for removal of the solvent by distillation in an oil bath maintained at 130°C in 2-3 min. The residue was continued to heat at the same temperature for an additional 30 min in an open system. After cooling, the mixture was dissolved in MeOH-H_2O (8:2) and injected onto a HPLC consisting of a reverse phase column [MeOH-H_2O (8:2), 4 ml/min] and a normal phase column [$CHCl_3$-MeOH (98:2), 4 ml/min] for final purification to give chemically and radiochemically pure (13). The isolated radiochemical yield of (13) in several experiments was in the range of 30-35%. The synthesis time was about 2 hr.

8-[^{131}I]Iodo-2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one (14)

Method A. A mixture of the triazene (10) (1 mg) and dry $Na^{131}I$ (2.43 mCi) in dry acetonitrile (2 ml) containing three equivalents of methanesulfonic acid was refluxed with constant stirring for 60 min. The progress of reaction was followed by TLC [reverse phase plates, MeOH-H_2O (8:2), R_f for 14 = 0.20]. After removal of the solvent, the residue was dissolved in $CHCl_3$-MeOH (97:3) and injected onto a HPLC normal phase column [$CHCl_3$-MeOH (97:3), 4 ml/min]. The collected fraction (164 µCi) of the radioiodinated compound (eluted after 37 min) was evaporated to dryness. The residue was dissolved in MeOH-H_2O (8:2) and the second purification performed on a reverse phase column [MeOH-H_2O (8:2), 4 ml/min] gave 132 µCi (5.4%) of (14) (eluted after 16 min) with no-impurities detectable by UV spectroscopy or radioactivity. The synthesis time required was about 2.5 hr.

Method B. Three hundreds µl of a CH_3CN solution (3 ml) containing methanesulfonic acid (2.7 mg) were added to a vial containing the triazene (10) (1 mg). The yellow suspension was subjected to an ultrasonic treatment for 30 sec. The resulting purple suspension was added directly to a test tube containing dry

$Na^{131}I$ (600 μCi-1 mCi). The procedure followed was essentially that used to prepare the compound (13) by method B. The product was purified by HPLC using the same columns and conditions as those described in method A to give chemically and radiochemically pure (14). Isolated radiochemical yields of 15-25% were obtained. The synthesis time was about 2 hr.

Partition coefficients

These were measured by mixing 0.1 μCi of the radioiodinated compounds into a vial containing 0.5 ml of 1-octanol and 0.5 ml of phosphate buffer (0.1 M) at the desired pH at 37°C, according to a procedure described by Kung et al (24). The measurement was repeated four times.

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