SYNTHESIS OF 9-[(2-CHLORO-6-FLUOROPHENYL)[^{14}C]METHYL]-9H-PURINE-6-AMINE (ARPRINOCID) AND 9-[(2,6-DICHLOROPHENYL)[^{14}C]METHYL]-9H-PURINE-6-AMINE

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SUMMARY

The synthesis of 9-[(2-chloro-6-fluorophenyl)methyl]-9H-purin-6-amine (arprinocid, a coccidiostat candidate) and 9-[(2,6-dichlorophenyl)methyl]-9H-purin-6-amine labeled with carbon-14 in the benzylic methylene carbon of each of these is described. Introduction of the carbon-14 label leading to the formation of the key radioactive intermediates, 2-chloro-6-fluoro[carboxyl-^{14}C]benzoic acid and 2,6-dichloro-[carboxyl-^{14}C]benzoic acid, was accomplished by utilizing [^{14}C]carbon dioxide for carbonation of a suitable dihalophenyl Grignard or organolithium reagent.

Key Words: 9-[(2-chloro-6-fluorophenyl)[^{14}C]methyl]-9H-purin-6-amine, arprinocid, 9-[(2,6-dichlorophenyl)[^{14}C]methyl]-9H-purin-6-amine, 2-chloro-6-fluoro[carboxyl-^{14}C]benzoic acid, ^{14}C-carbonation, haloaromatic Grignard reagents.

INTRODUCTION

Labeled arprinocid, 9-[(2-chloro-6-fluorophenyl)methyl]-9H-purine-6-amine, a compound being studied for development as a coccidiostat (1), and the 2,6-dichloro analog were needed for comparative study. The planned residue and metabolism studies (2) required large quantities of ^{14}C-labeled drugs at high specific activity. Methods were developed for preparing each of these compounds with carbon-14 in a specific position such that

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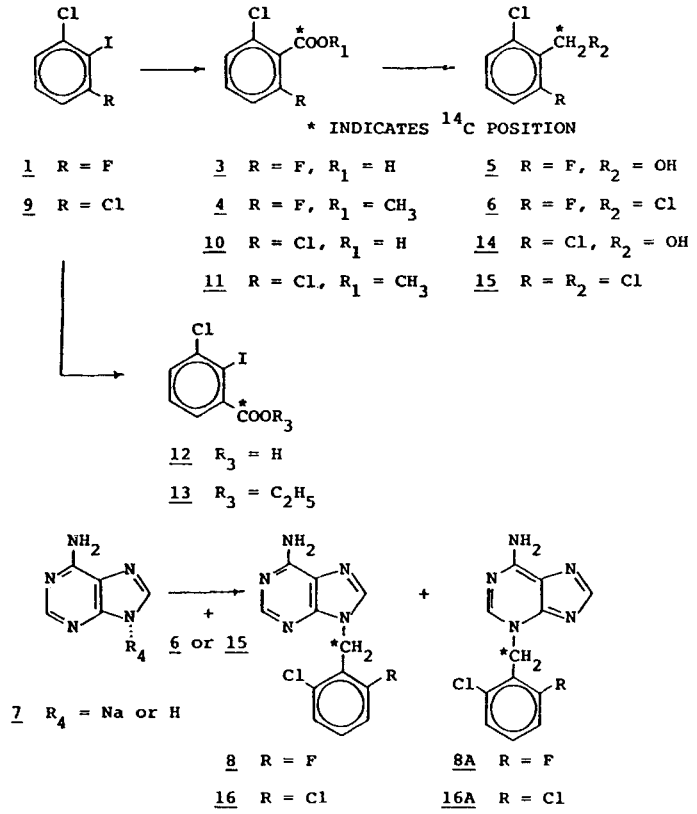
meaningful residue and metabolism studies could be done with the tracers. Choice of [^{14}C]carbon dioxide to react with dihalophenyl Grignard or dihalophenyllithium reagents for introduction of the radioactive label provided a convenient and direct route to the compounds and afforded tracers with the high specific activity near that for one pure carbon-14 atom per molecule.

The reaction scheme shows the formation of 2-chloro-6-fluoro- and 2,6-dichloro[carboxyl-^{14}C]benzoic acids (3) and (10) from radioactive carbon dioxide, and the conversions of these substances to the corresponding title compounds (8) and (16).



DISCUSSION

Treatment of an organomagnesium halide with carbon dioxide to obtain a carboxylate was described in the earliest reported

studies of Grignard (3). It was soon discovered (4) that carbonation of mono-Grignard reagents derived from dihalobenzenes under controlled conditions could lead to halobenzoic acids in substantial yields. The order of reactivity of aromatic halogen with magnesium for Grignard reagent formation or with n-butyllithium for translithiation was found to be: I>Br>Cl>F (5). This permits selectivity in the site of metalation, whence subsequent carbonation can lead predominately to the expected halobenzoic acid. The relatively greater stability of fluorine in fluorohalophenyl compounds led to the study of fluorophenylmagnesium halides and fluorophenyllithium reagents (6), including carbonation of these to yield fluorobenzoic acids (6,8,9). Such procedures have been utilized with radioactive carbon dioxide to prepare carboxyl-^{14}C labeled halobenzoic acids (6,7,10,11,12). In the examples studied here carbonation of 2,6-dichlorophenylmagnesium iodide and 2-chloro-6-fluorophenyllithium led predominately to the expected and desired halobenzoic acids. The starting trihalobenzenes (1) and (9) for this study were derived from the readily available unlabeled acids (3) and (10). These were converted to the corresponding anilines using the procedure of Roe (13), followed by diazotization and treatment with potassium iodide to obtain (1) and (9).

The reported instability of o-fluorophenyl Grignard reagents (12) was confirmed in our laboratory by our inability to successfully prepare 2-chloro-6-fluoro[carboxyl-^{14}C]benzoic acid (3) by carbonation of the Grignard reagent derived from 1-chloro-3-fluoro-2-iodobenzene (1). Under the literature conditions (12,14) for exchange with n-butyllithium to form 2-chloro-6-fluorophenyllithium followed by carbonation with $^{14}CO_2$ at -78°C we were able to achieve a quantitative incorporation of carbon-14 dioxide. Careful GC-MS analysis of the methyl ester derived from the crude reaction product by reaction with diazomethane indica-

ted that the sequence had proceeded with excellent specificity, resulting in only the desired acid (3). The ester (4) was in turn reduced with Vitride^{R} to yield 2-chloro-6-fluoro[7-^{14}C]benzyl alcohol (5). Crude (5) dissolved in ether and without isolation was converted quantitatively to 2,7-dichloro-6-fluoro-[7-^{14}C]toluene (6) of >99% radioactive purity by distillative concentration in the presence of 12 N hydrochloric acid.

Our finding that 1,3-dichloro-2-iodobenzene was sufficiently reactive to form a stable Grignard reagent under mild (refluxing ether) conditions permitted the use of this substrate for introduction of the ^{14}C label. By this route a 96% incorporation of [^{14}C]carbon dioxide was achieved. This compares favorably with the result reported by Neish (11) who carried out the carbonation at -70°C on the mixture obtained from 2-bromo-1,3-dichlorobenzene and n-butyllithium to obtain (10) in 76% yield. The somewhat lower selectivity at the higher carbonation temperature (25°C) employed with the Grignard reagent resulted in the formation of about 5% of 3-chloro-2-iodo[carboxyl-^{14}C]benzoic acid as a contaminant. Esterification of the crude reaction mixture with ethanolic hydrogen chloride gave only ethyl 3-chloro-2-iodo[carboxyl-^{14}C]benzoate (13) as product, and allowed the more sterically hindered 2,6-dichloro[carboxyl-^{14}C]benzoic acid (10) to be isolated in pure form by sodium bicarbonate extraction in a net radiochemical yield of 91%. The free acid (10) reacted with diazomethane in ether to yield the corresponding methyl ester (11). Dilution of the crude radioactive ester with carrier ester and purification by preparative GC resulted in a 72% yield of radiochemically pure product. Reduction in this case was done with lithium aluminum hydride in THF at 25°C. Conversion of the resulting benzyl alcohol (14) to 2,6,7-trichloro[7-^{14}C]toluene (15) as described for (6) proceeded smoothly yielding radiochemically pure product in 97% yield.

A comment in the paper by Shinkai et al (15), in which the use of phase transfer catalysis for alkylation of sodium adeninate with (6) is described, that an optimum ratio of the 9- and 3- arprinocid isomers might be obtained when dimethyl sulfoxide is employed as a single phase reaction medium led to our choice of this simpler procedure for our preparation of (8). Accordingly the benzyl chloride (6) dissolved in dry dimethyl sulfoxide was found to react completely in four hours at 25°C with one equivalent of sodium adeninate mono-hydrate (7). The crude reaction product was shown by TLC analysis to be a mixture of the 9- and 3- isomers (8 & 8A) in the somewhat disappointing ratio of 4.26:1.0. Nearly all of the unwanted isomer was removed by recrystallization from water:acetic acid (3.5:1) to give a tracer acceptable for most of the planned studies. The over-all yield of arprinocid (8), specific activity: 89.9 μCi/mg, was 29.0 mCi (58% from [^{14}C]carbon dioxide). A particular experiment required labeled arprinocid with less than 50 ppm of the undesired 3-isomer (8A) for critical tissue residue studies. Preparation of such material was achieved by using the method described by Weinstock et al (16), i.e. selective destructive removal of the (8A) by warming the nearly acceptable product (8 & 8A) in a mixture of concentrated sulfuric acid and toluene at 55°C for eighteen hours. That complete removal of (8A) was achieved was shown by HPLC with liquid scintillation counting of the appropriate elution zones.

A mixture of 2,6,7-trichloro[7-^{14}C]toluene (15) and adenine (7) with potassium t-butoxide in ethanol was refluxed for five days to give a 94% combined yield of adducts (16) and (16A). Purification by recrystallization as described for (8 + 8A) removed the 30% of 3-isomer (16A) to give tracer quality (>99% radiochemical purity) 9-[(2,6-dichlorophenyl)[^{14}C]methyl]-9H-purine-6-amine (16). For this series the overall radiochemical yield from [^{14}C]carbon dioxide was 47%.

EXPERIMENTAL

Analytical TLC was carried out on 5 x 20 cm glass plates precoated with silica gel 60 F-254 (E. Merck, Darmstadt, Germany). Radioactive zones were located with a Berthold Model LB2760 scanner. For final establishment of radioactive purity the total radioactivity in each zone was determined by scraping and scintillation counting of zones from the developed plates. A Packard Tri-Carb Model 3320 liquid scintillation spectrometer with 0.4% Omnifluor^{R} in toluene:ethanol (7:3) as scintillation medium was used for all radioactivity measurements. The purity and specific activity of the starting [^{14}C]carbon dioxide was taken as stated by the supplier (American Radiochemical Corp.).

1-Chloro-3-fluoro-2-iodobenzene (1) - - While stirring at room temperature a solution of 10.9 g (75 mmoles) of 2-chloro-6-fluoroaniline in 250 ml of glacial acetic acid was treated with 6.5 ml of 12 N hydrochloric acid. The resulting slurry was cooled to 5°C and a solution of 5.45 g (79 mfw) of sodium nitrite in 10 ml of water was then added dropwise over a period of 30 minutes while keeping the temperature in the range 5-10°C. The mixture was aged for one hour at 5-10°C after this addition was completed, then a cold solution of 25.7 g (155 mfw) of potassium iodide in 25 ml of water was added as rapidly as possible (some foaming occurred during this addition). Stirring was continued until the foaming subsided and the mixture was then allowed to warm to room temperature and stand for eighteen hours. The mixture was then poured into 500 ml of water and the product (1) was extracted into hexane. Standard work up procedure with vacuum distillation gave 9.0 g (35.1 mmoles) of pale yellow liquid product, b.p. 95-97°C/7 mm. Analysis of this distillate by GLC (3% OV-1, 6 ft., 100°C) showed a single component: MS m/e 256,258 (M^+), 129,131 (M^+ - I).

1,3-Dichloro-2-iodobenzene (9) - - The dichloro analog (9) was prepared in a similar manner. The crude product was first crystallized from hexane, then from ethanol to give (9), mp 67-68°C (lit. (17) mp: 68°C) in 65% yield.

2-Chloro-6-fluoro[carboxyl-^{14}C]benzoic acid (3) - - A solution of 770 mg (3.0 mmoles) of freshly distilled 1-chloro-3-fluoro-2-iodobenzene (1) in 30 ml of dry ether was cooled to -78°C under nitrogen and treated at that temperature for 5 minutes with 2.96 mmoles of n-butyllithium. This reaction mixture was then cooled to liquid nitrogen temperature and 50 mCi of [^{14}C]carbon dioxide (59.1 mCi/mmole) along with 74.8 mg of carrier carbon dioxide was added using standard vacuum transfer procedure. The mixture was allowed to warm to -78°C and aged for (exactly) fifteen minutes. The resulting slurry was quenched while still at -78°C with first 10 ml of water and then with 1 ml of 2.5 N sodium hydroxide. After warming to 0-5°C the ether layer was removed. The aqueous layer was acidified with dilute hydrochloric acid and the liberated halobenzoic acid then extracted into ether. The ether extract was dried over anhydrous magnesium sulfate, filtered, and concentrated under nitrogen to a constant weight residue. The product thus obtained weighed 432.2 mg (97.1% of theory on total carbon dioxide) and was >99% radiochemically pure by TLC analysis (silica gel, $CHCl_3$:MeOH, 3:1). The specific activity was 116.8 μCi/mg, (leading to 50.5 mCi total radioactivity, quantitative).

Methyl 2-chloro-6-fluoro[carboxyl-^{14}C]benzoate (4) -- To 432 mg (2.47 mmoles) of 2-chloro-6-fluoro[carboxyl-^{14}C]benzoic acid (3) dissolved in 25 ml of ether was added in small portions freshly prepared diazomethane in ether until TLC analysis (silica gel, ether) showed complete conversion of the starting (3) to methyl ester (4). Removal of the ether *in vacuo* at < 25°C gave 555 mg

of (4) with 50 mCi of carbon-14. TLC (silica gel, hexane:ether, 1:1) showed by radioscan that 95.5% of the plate radioactivity was in the product (4) zone. GC-MS analysis of this ester confirmed that the carbonation had proceded exclusively at the position originally occupied by iodine in (1) i.e. the product was methyl 2-chloro-6-fluoro[carboxyl-^{14}C]benzoate (4) as expected.

2-Chloro-6-fluoro[7-^{14}C]benzyl alcohol (5) -- To a solution of 555 mg (92.93 mmol, 50 mCi) of methyl 2-chloro-6-fluoro[carboxyl-^{14}C]benzoate (4) in 25 ml of dry THF was added, under nitrogen at 25°C and over five minutes, a solution of 3700 mg of Vitride^{R} (70% sodium bis-(2-methoxyethoxy)aluminum hydride in benzene). After aging at 25°C for 1/4 hour the reaction mixture was quenched with water, then with 6 N hydrochloric acid to strong acidity, and the product (5) extracted into ether. Analysis of the ether extract by TLC (silica gel, ether:hexane, 1:1) indicated essentially pure (5). The solution containing this product ((5) 50 mCi) was used as indicated for conversion to (6).

2,7-Dichloro-6-fluoro[7-^{14}C]toluene (6) -- To the ether solution of (5) (see above) was added 10 ml of 12 N hydrochloric acid. The ether was removed by distillation until the temperature of the remaining aqueous layer came to 95°C. After aging at that temperature for four hours, the mixture was cooled to 25°C and the product was extracted into ether. The ether extract was dried over anhydrous magnesium sulfate and filtered. Upon concentration of the filtrate there was obtained 514.9 mg (98.3%) of (6), an oil. TLC (silica gel, ether:hexane, 1:1) showed by radioscan that 99.9% of the plate radioactivity was in the product (6) zone.

2,6-Dichloro[carboxyl-^{14}C]benzoic acid (10) -- To a mixture of 139 mg (5.7 matoms) of magnesium turnings and 5 ml of ether was

added a solution of 60 mg (0.3 mmol) of 1,2-dibromoethane in 1.0 ml of ether. After ten minutes of refluxing under nitrogen a solution of 1250 mg (4.6 mmoles) of 1,3-dichloro-2-iodobenzene (9) in 10 ml of ether was added dropwise over a one hour period. The mixture was refluxed for two hours after completion of this addition, then was diluted with ether to a final volume of 35 ml. After cooling to liquid nitrogen temperature, 122.4 mg (2.78 mmoles, 10 mCi/mmol, 27.8 mCi) of [^{14}C]carbon dioxide was added using standard vacuum transfer procedure. The mixture was warmed to 25°C and kept at that temperature for sixteen hours. The resulting slurry was quenched with 10 ml each of water and ether, then 2.5 N hydrochloric acid was added until the aqueous layer was acidic to Alkacid^{R} paper. The ether layer was then removed and the aqueous layer extracted with 3 x 25 ml portions of ether. The combined ether and extracts were then extracted with aqueous sodium bicarbonate solution. This extract was acidified and the liberated product was extracted into ether. The ether solution was dried with anhydrous magnesium sulfate, filtered, and concentrated to yield 550 mg of crude product. GC-MS of the crude isolate indicated the presence of about 5% of 3-chloro-2-iodo-[carboxyl-^{14}C]benzoic acid (12) along with the major product (10). This crude mixture was dissolved in 25 ml of saturated ethanolic hydrogen chloride. Benzene (25 ml) was added and the solution was concentrated *via* atmospheric distillation to a final volume of 1-2 ml. This procedure was repeated and the concentrate was then quenched with 10 ml of water. The aqueous layer was made alkaline by the addition of solid sodium bicarbonate. Extraction with ether gave, after the usual work-up, 48.3 mg (5.6%) of ethyl 3-chloro-2-iodo[carboxyl-^{14}-C]benzoate (13). Upon acidification the bicarbonate solution yielded 484 mg (91.2%) of (10).

Methyl 2,6-dichloro[carboxyl-^{14}C]benzoate (11) -- The procedure used was identical to that described for the preparation of (4). From 484 mg (2.53 mmoles) of (10) there was obtained 510 mg of crude (11). Dilution of this with unlabeled carrier (11) and purification by preparative GLC (5% SE-30, 220°C) gave 460 mg (72%) of >99% radiochemically pure (11).

2,6-Dichloro[7-^{14}C]benzyl alcohol (14) -- To a solution of 460 mg (2.24 mmoles) of methyl 2,6-dichloro[carboxyl-^{14}C]benzoate (11) in 20 ml of dry THF was added in one portion, under nitrogen, 5 ml of a 0.47 M solution of lithium aluminum hydride in THF. The reaction mixture was stirred at 25°C for three hours, then was quenched with sufficient water to cause the separation of a gum from a clear supernatent. An excess (several grams) of anhydrous magnesium sulfate was added. The mixture was stirred for fifteen minutes, then was filtered. The collected solids were washed well with ether and the combined ether solution was concentrated under reduced pressure to yield 393 mg (99%) of pure (14).

2,6,7-Trichloro[7-^{14}C]toluene (15) -- Following the procedure described for the preparation of (5) 393 mg of 2,6-dichloro[7-^{14}C]benzyl alcohol (14) was dissolved in ether and treated with concentrated hydrochloric acid to provide 420 mg (96.8%) of (15) which crystallized to a solid mass upon standing. TLC analysis (ether, silica gel) with radioscan indicated that this material was 99% radiochemically pure.

Sodium adeninate hydrate (7) -- A solution of 10.44 g (75 mmoles) of adenine in 25 ml of 3.0 M sodium hydroxide solution was evaporated to dryness, then dried to constant weight at 0.5 mm and 100°C. The yield of this product was quantitative.

9-[(2-Chloro-6-fluorophenyl)[^{14}-C]methyl]-9H-purin-6-amine (8), (arprinocid) -- To a stirred mixture of 504 mg (2.88 mmoles) of sodium adeninate monohydrate (7) and 2.0 ml of dry dimethyl sulfoxide (DMSO) was added, over a period of one hour, a solution of 514.9 mg (2.88 mmoles) of 2,7-dichloro-6-fluoro[7-^{14}C]toluene (6) in 1.0 ml of dry DMSO. Following this addition, any remaining (6) was rinsed in with an additional 2 x 1.0 ml of DMSO, and the resulting slurry was aged for four hours at 25°C. The mixture was then quenched with 15 ml of water and the precipitated solid was removed by filtration. After washing the solid well with cold water and then drying, a yield of 572 mg of product was obtained. TLC (silica gel, developed with benzene: methanol, 1:1) with radioscan and integration of radioactive zones indicated that 74.1% of the plate radioactivity was associated with [^{14}C]arprinocid (8). Removal of the isomeric by-product (8A) was accomplished by dissolution of the crude isolate in 1.5 ml of hot glacial acetic acid and addition of this solution, along with 2 x .75 ml warm acetic acid rinses, dropwise to 10 ml of water at 90°C over a period of five to ten minutes. The mixture was then slowly cooled (thirty minutes) to 50°C and the resulting slurry was immediately filtered. The collected solid was washed well with cold water and dried to constant weight to yield 323 mg (40.4%) of [^{14}C]arprinocid (8). The overall yield from [^{14}C]carbon dioxide was 29.0 mCi. The specific activity of this lot was 89.9 μCi/mg.

Destructive removal of (8a) from arprinocid (8) -- A mixture of 716 mg (2.6 mmoles, 205 μCi/mg, 56.9 mCi/mmole; this lot was prepared from high specific activity $^{14}CO_2$ without the addition of carrier CO_2) of nearly pure [^{14}C]arprinocid (8), 1.5 ml of 36 N sulfuric acid, and 1.5 ml of toluene was heated and vigorously stirred at 55°C for eighteen hours. The mixture was then cooled in an ice bath and 5 ml of water was added. The precipitated

solids were redissolved by heating to 90°C and the upper toluene layer was removed by decanting. The aqueous layer was extracted while still hot with 3 x 3 ml portions of toluene, then cooled to 0 - 5°C and made alkaline by the addition of concentrated ammonia. The resulting slurry was filtered and the collected solid was washed well with cold water and dried. The recovery was 692 mg (96.6%). The two materials (8 & 8A) were quite well separated by HPLC analysis using a Partisil^{R} ODS-2 column (Whatman) with a mobile phase of 0.02 M KH_2PO_4 (pH 3.0):CH_3OH, 55:45, at a flowrate of 1.0 ml/min. Visualization (UV @ 254 nm) of the 3-isomer (8A) area of interest was achieved by spiking the the analytical sample with authentic reference (8A) prior to injection onto the column. The radioactivity profile, determined by collection of one minute fractions followed by liquid scintillation counting, indicated that the amount of radioactive (8A) remaining was 2 ppm or less.

9-[(2,6-Dichlorophenyl)[^{14}C]methyl]-9H-purin-amine (16) -- A mixture of 290 mg (2.15 mmoles) of adenine (7), 420 mg (2.15 mmoles) of 2,6,7-trichloro[7-^{14}C]toluene (15), 303 mg (2.7 mmoles) of potassium t-butoxide, and 7.5 ml of anhydrous ethanol was refluxed under an atmosphere of nitrogen for five days. Most of the ethanol was removed in vacuo and the residue was suspended in 10 ml of water. Filtration of the resulting slurry gave 592 mg (93.6%) of crude product. Two recrystallizations from 10 vol. (each) of water:acetic acid (4:1) resulted in complete removal of the approximately 30% of the 3-isomer (16A), and yielded 392 mg (49.4%) of radiochemically pure (16) (TLC: silica gel, benzene:methanol, 1:1). The specific activity of the resulting product was 21.4 Ci/mg. The overall radiochemical yield from [^{14}C]carbon dioxide was 47%.

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