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SYNTHESIS OF CARBON-14 AND SULFUR-35 LABELED CLORSULON

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SUMMARY

Preparation of the title compounds, tracer substances required for the development of the fasciolide clorsulon, is described. The ring-u-^{14}C variety was obtained in 42.2% yield overall from purchased [ring-u-^{14}C]acetophenone.

Key Words: [^{14}C]clorsulon, [^{35}S]clorsulon, radiolabeled fasciolides, 4-amino-6-(trichloroethenyl)-1,3-benzenedisulfonamides, 4-amino-6-(trichloroethenyl)-1,3-[ring u-^{14}C]benzenedisulfonamide.

INTRODUCTION

Residue and metabolism studies (2,3) relevant to the development of clorsulon [MK-0401, 4-amino-6-(trichloroethenyl)-1,3-benzenedisulfonamide (9)], a new and potent fasciolide (1), required substantial quantities of radiolabeled tracer. Material labeled with carbon-14 in the benzene ring of the molecule was finally selected as best suited for the critical residue studies. This paper describes the synthesis of this (9a), as well as some of our earlier work which led to other labeling patterns.

DISCUSSION

[7-^{14}C]Acetophenone (2b) was prepared by reaction of benzene with [1-^{14}C]acetyl chloride generated *in situ* from sodium [1-^{14}C]-acetate upon the addition of aluminum chloride to a suspension of the salt in benzene (4). Procedures used in the following steps were based largely largely on the work described in reference (1)

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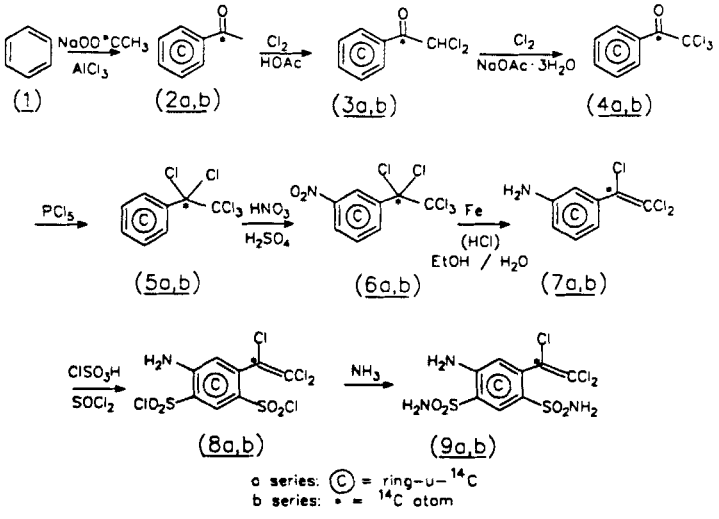
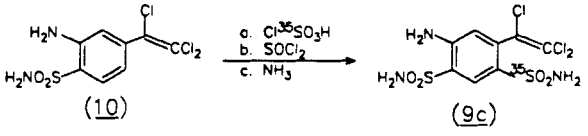
[^{14}C]CICLORSULON SYNTHESIS SCHEME



[^{35}S]CICLORSULON SYNTHESIS SCHEME



and references cited therein. The acetophenones (2a & 2b) reacted with chlorine in acetic acid to give the dichloro intermediates (3a and 3b). Conversion to the trichloro compounds (4a & 4b) was accomplished by continued chlorination after addition of fused sodium acetate to the solutions of crude (3a & 3b) (5). The trichloroacetophenones (4a & 4b) were converted to the pentachloroethyl intermediates (5a & 5b) by prolonged heating with phosphorus pentachloride. Timely removal of the phosphorus oxychloride formed in this reaction was shown to be important in driving this reaction to completion. Nitration of these intermediates (5a & 5b) under standard nitrating conditions led to the meta nitro intermediates (6a & 6b). Reduction of the nitro function with iron in acidic ethanol-water led to the elimination of two chlorine atoms from the side chain as well as generation of the expected amino function. Treatment of the resulting

anilines (7a & 7b) with chlorosulfonic acid followed by thionyl chloride gave the disulfonyl chlorides (8a & 8b). For preparation of the analogous sulfur-35 labeled tracer (9c) 2-amino-4-(trichloroethenyl)benzenesulfonamide (10) (1) was caused to react with chloro[^{35}S]sulfonic acid. The observed specific activity of the final product in this instance, 4-amino-6-trichloroethenyl-1,3-benzenedi[^{35}S]sulfonamide (9c), indicated that little if any equilibration with the sulfonamido group of the precursor (10) occurred in this step. The desired final products (9a, 9b, 9c) were readily obtained by treatment of the chlorosulfonyl intermediates with gaseous ammonia. Purification to quality suitable for tracer use was achieved by recrystallization.

EXPERIMENTAL

Analytical TLC was carried out on 5x20 cm glass plates pre-coated with silica gel (60 F-254 E. Merck, Darmstadt, Germany). Radioactive zones were located with a Berthold Model LB2722 scanner. Radioactivity was determined with a Packard Tri-Carb^{R} Model 3320 liquid scintillation spectrometer, using 0.4% OMNIFLUOR^{R} in toluene/ethanol (7:3) as scintillation medium. Purity and specific activity of the radioactive starting materials: [ring-u-^{14}C]acetophenone (2a) (Pathfinder), sodium [1-^{14}C]acetate (ICN) and chloro[^{35}S]sulfonic acid (Amersham), were assumed to be correct as stated by the suppliers. Identities of the intermediates and final tracer products were established by comparison with authentic reference samples of the unlabeled substances.

[7-^{14}C]Acetophenone (2b). -- To insure dryness 5 ml of benzene was distilled from a suspension of 744 mg, 9.0 mmol, specific activity 21.44 mCi/mmol, 193 mCi, of sodium [1-^{14}C]acetate in 10 ml of that solvent. To this suspension, while stirring at room temperature under nitrogen, was then added 6150 mg, 46 mfw, of anhydrous

aluminum chloride. After a brief surge of gaseous HCl the mixture remained quiescent as it was heated to 90°C and stirred at that temperature for 8 hours. After cooling to room temperature (overnight) the mixture was poured onto 30-40 g of ice, and the crude product was extracted into ether. After drying ($MgSO_4$) the ether and any residual benzene were removed by distillation through a small helices-filled column at atmospheric pressure. The residual crude (2b) was distilled at 60°C/10mm, followed by a system flush with 1.0 g of carrier acetophenone to provide a total of 2180 mg, 18.15 mmol, of [7-^{14}C]acetophenone (2b), specific activity 10.0 mCi/mmol, 181.5 mCi, a radiochemical yield of 94% on the starting [^{14}C]acetate.

8,8-Dichloro[7-^{14}C]acetophenone (3b). -- Chlorine was passed into a solution containing 2180 mg (18.15 mmol, specific activity 10.0 mCi/mmol, 181.5 mCi) of [7-^{14}C]acetophenone (2b) in 9 ml of glacial acetic acid while the mixture was stirred in an oil bath at 55-60°C. After five hours glc evaluation (4' column, with silicone R-gum SE-30, on Chromosorb P, at 170°C) indicated that conversion to the dichloroacetophenone (3b) was complete.

8,8-Dichloro[ring-u-^{14}C]acetophenone (3a). -- In exactly the same manner as described for (3b) 1682 mg, 14.0 mmol, specific activity 17.76 mCi/mmol, 248.6 mCi, of [ring-u-^{14}C]acetophenone (2a) was chlorinated to furnish (3a). As with (3b), this intermediate was carried on to (4a) without isolation.

8,8,8-Trichloro[7-^{14}C]acetophenone (4b). -- After flushing the solution of (3b) for 45 minutes at 50°C with nitrogen 2731 mg (19.9 mmol) of fused sodium acetate was added, and chlorine addition with heating at 50°C was continued for three additional hours. Glc (under conditions as described above) then showed essentially complete conversion to the trichloroacetophene (4b). After cooling, the reaction mixture was poured into a solution containing 240 mg of sodium bisulfite in 27 ml of water.

Extraction with ether (4 x 20 ml) gave a solution which, after washing with water and drying, was concentrated to leave 5482 mg of a liquid. This crude product was vacuum distilled at 80°C/0.4-0.5 mm, to provide 4331 mg of 8,8,8-trichloro[7-^{14}C]acetophenone (4b), (6.5% overweight for theoretical yield).

8,8,8-Trichloro[ring-u-^{14}C]acetophenone (4a). -- Addition of 2161 mg of freshly fused sodium acetate to the solution of (3a) and continued chlorination as described for (3b) led to 6057 mg of crude (4a). Vacuum distillation of this yielded 3857 mg of product with a specific activity of 7.1 μCi/mg, for a total of 274 mCi, suggesting that the supplier's statement of radioactive precursor supplied was a bit low.

1,1,1,2,2-Pentachloro-2-phenyl[2-^{14}C]ethane (5b). -- A mixture of 4333 mg, 19.8 mmoles, of 8,8,8-trichloro[7-^{14}C]acetophenone (4b) and 4652 mg, 22.4 mmol, of phosphorus pentachloride was heated to reflux and stirred in an oil bath at 215-225°C for nine hours, then an additional 4449 mg of this reagent was added, and heating under reflux was continued for an additional 22 hours. Glc analysis at this point indicated that only 30% of the (4b) had been converted to (5b). A third charge of 7662 mg, 34.9 mmol, of PCl_5 was added and reflux continued for an additional 24 hours. Conversion was then only 85% complete. About 4 ml of the liquid (mostly $POCl_3$) was then allowed to distill over, and a final 4 hours of reflux gave a reaction mixture with essentially a single glc peak, that for (5b). After cooling to room temperature the mixture was quenched by pouring into 60 g of ice and water. The quench suspension was stirred for 45 minutes, then the product was extracted into ether. Usual work up with final vacuum distillation (90°C/0.3 mm) gave 4774 mg, 17.1 mmol, 86.2%, of 1,1,1,2,2-pentachloro-2-phenyl[2-^{14}C]ethane (5b), with a specific activity of 9.66 mCi/mmol, a total of 164.8 mCi.

1,1,1,2,2-Pentachloro-2-[ring-u-^{14}C]phenylethane (5a). -- In a

manner similar to that described for (5b), but with $POCl_3$ removed as formed in the earlier stages of the reaction thus permitting a shorter total reaction time, 3856 mg, 13.95 mmol, of (4a), with a total of 8000 mg of phosphorus pentachloride (added in several portions) gave 4218 mg, 15.2 mmol (over theory), of (5a). This crude product was carried on in the sequence without distillation.

3-(Pentachloro[1-^{14}C]ethyl)nitrobenzene (6b). -- To a mixture of 2.86 g of conc. nitric acid and 6.20 g of conc. sulfuric acid was added 4767 mg, 17.1 mmol, of the pentachloro[1-^{14}C]ethylbenzene (5b), rinsing in any residual substrate with 4 ml additional conc. H_2SO_4. The resulting mixture was stirred for four hours at 35°C, during which time an oil began to separate and crystallize. The mixture was cooled in an ice bath, poured into a mixture of 60 g of ice and water and 20 ml of methylene chloride. Usual extractive work up gave 6141 mg of crude product. Crystallization from methanol gave 4221 mg, 13.1 mmol, 76.2%, of 3-(pentachloro-[1-^{14}C]ethyl)nitrobenzene (6b). The specific activity of this material was 10.34 mCi/mmol, a total of 135 mCi. TLC analysis showed a single (radioactive) spot.

3-(Pentachloroethyl)nitro[ring-u-^{14}C]benzene (6a). -- As described for (6b) 4128 mg of pentachloroethyl[ring-u-^{14}C]benzene (5a) was treated with the nitric/sulfuric acid mixture to yield 4633 mg (> theory) of crude (6a). This product was crystallized from methanol to provide 3583 mg, 11.1 mmol, of purified (6a) with a specific activity of 55.4 μCi/mg, 17.9 mCi/mmol, 79.4% (radiochemical) yield.

3-(Trichloro[1-^{14}C]ethenyl)aniline (7b). -- To a mixture of 4221 mg, 13.1 mmol, of 3-(pentachloro[1-^{14}C]ethyl)nitrobenzene (6b) and 8013 mg, 143.5 m.a.w., of iron powder (B & A reagent, reduced, code 1809) in 130 ml of 50% (v/v) ethanol/water warmed to 50°C was added dropwise 1.7 ml of a solution of 2 N HCl in 50% (v/v) aqueous ethanol. The resulting mixture was heated to reflux in an

oil bath for 90 minutes and then filtered while hot through a filter aid pad. The filter cake was washed 3x70 ml with hot 50% (v/v) aqueous ethanol followed by 70 ml of hot ethanol. The combined filtrate and washes were neutralized with 100 ml (excess) of saturated aqueous $NaHCO_3$. The product was extracted from the resulting suspension with methylene chloride. After washing with 5x75 ml of saturated aqueous NaCl and drying over Na_2SO_4 the solution (which had become slightly orange in color during the overnight drying) was filtered, and the solvent removed to leave 2925 mg, 13.1 mmol, 100%, of 3-(trichloro[1-^{14}C]ethenyl)aniline (7b), specific activity 10.15 mCi/mmol, a total of 133 mCi.

3-(Trichloroethenyl)[ring-u-^{14}C]aniline (7a). -- In a manner exactly as described for (7b) 3580 mg of (6a) was caused to react with iron powder in aqueous ethanol-HCl to yield 2466 mg, 11.08 mmol, of 3-(trichloroethenyl)[ring-u-^{14}C]aniline (7a), specific activity 86.4 μCi/mg (about 7% higher than expected), a total of 213 mCi.

4-Amino-6-(trichloro[1-^{14}C]ethenyl)-1,3-benzenedisulfonyl chloride (8b). -- To 2925 mg of 3-(trichloro[1-^{14}C]ethenyl)aniline (7b) was added dropwise 4.6 ml of chlorosulfonic acid, while cooling to keep the mixture below 10°C and stirring the resulting very viscous gummy mass manually (addition time ca 5 minutes). Upon heating in an oil bath at 130-135°C the resulting mixture soon softened to a dark, thick syrup which was then stirred mechanically for 2 hours. At this point TLC showed no evidence of remaining starting material. The mixture was then cooled to about 60°C and 3.3 ml (5.4 g, 45 mmol) of thionyl chloride was slowly added. After the considerable initial foaming had subsided the mixture was heated to reflux (bath temp 105°C) for 2 hours. TLC at this stage was complicated by what appeared to be partial conversion to the sulfonic acid upon spotting, however there was no evidence of any remaining starting material (7b). After

cooling the crude disulfonyl chloride (8b) reaction mixture was quenched by transfering into a mixture of ice and methylene chloride. Removal of the solvent after extraction, washing and drying (Na_2SO_4) left 5437 mg, 12.9 mmol, 95 % of 4-amino-6-(trichloro[1-^{14}C]ethenyl)-1,3-benzenedisulfonyl chloride (8b) as a brown foam, specific activity 9.7 mCi/mmol, 125 mCi.

4-Amino-6-(trichloroethenyl)-1,3-[ring-u-^{14}C]benzenedisulfonyl chloride (8a). -- In a manner much as described for (8b), but using ethyl acetate in the work up to replace methylene chloride, 2800 mg, 10.81 mmol, of 3-(trichloroethenyl)[ring-u-^{14}C]aniline was transformed to 4-amino-6-(trichloroethenyl)-1,3-[ring-u-^{14}C]-benzenedisulfonyl chloride (8a). The resulting solution of crude (8a) was carried on into the final amidation step without isolation or characterization of the intermediate.

4-Amino-6-(trichloro[1-^{14}C]ethenyl)-1,3-benzenedisulfonamide (9b). -- Ammonia (gas) was passed through a solution containing 5437 mg, 12.9 mmol, of 4-amino-6-(trichloro[1-^{14}C]ethenyl)-1,3-benzene-disulfonyl chloride (8b) in 54 ml of methylene chloride for 15 minutes while cooling and stirring in a Dry Ice/acetone bath. The bath was removed, and as the resulting mixture warmed to room temperature and the excess ammonia evaporated over a 1 hour period the product slowly precipitated, with any gummy material quickly crystallizing upon scraping with a spatula. Nitrogen was bubbled through while the resulting slurry was stirred at room remperature for about 1 hour. The crude solid product was collected, air dried, and partitioned between ethyl acetate and water (30 ml each), thus removing entrapped ammonium chloride. The EtOAc layer was separated and the aqueous layer extracted with additional EtOAc. The combined EtOAc solution was washed with dilute aqueous NaCl, dried (Na_2SO_4), and concentrated to a volume of about 6 ml. After the addition of 2 ml of hexane and seeding with reference (9) the product separated as a thick slurry. After the addition

of 5 ml of a 20/7 mixture of EtOAc and hexane the slurry was stirred for an additional hour in an ice bath. The resulting material was collected and washed with 2 ml of 20/7 EtOAc/hexane followed by 5 x 2 ml of 1/1 EtOAc/hexane. After air drying the off-white solid product weighed 2650 mg. Radioscanning of a TLC plate at this stage showed the presence of about 2 % of a radioactive impurity.

The product was further purified by dissolving in 13.4 ml of a 17/3 (v/v) water/methanol mixture at reflux. After refluxing for ca. 3/4 hour the solvent was allowed to boil off until precipitation became heavy. (This treatment caused break up of an EtOAc complex and removal of the resulting free EtOAc.) The residual slurry was stirred vigorously for 1 hour in an ice bath, then the product was collected, washed, and air dried to yield 2209 mg, 5.8 mmol, 83% purification/recrystallization recovery, 44.8% from the (8b). TLC purity of the final material as measured in 2 systems was 98.82-98.91 %, with no discrete impurity detectable. Specific activity of the 4-amino-6-(trichloro[1-^{14}C]-ethenyl)-1,3-benzenedisulfonamide (9b) so prepared was 10.12 mCi/mmol, for a total of 58.9 mCi.

4-Amino-6-(trichloroethenyl)-1,3-[ring-u-^{14}C]benzenedisulfonamide (9a). -- In a manner similar to that described for (9b) the EtOAc solution of 4-amino-6-(trichloroethenyl)-1,3-[ring-u-^{14}C]benzene-disulfonyl chloride (8a) was treated in that solvent with gaseous ammonia, isolated, purified and recrystallized to provide 2027 mg, 5.32 mmol, of 4-amino-6-(trichloroethenyl)-1,3-[ring-u-^{14}C]ben-zenedisulfonamide (9a), specific activity 52 μCi/mg, 19.8 mCi/mmol, a total of 105.4 mCi. This represents a radiochemical yield of 88% from (8a), 49.2% from (6a), and 42.6% overall from the purchased [ring-u-^{14}C]acetophenone (2a).

4-Amino-6-trichloroethenyl-1,3-benzenedi[^{35}S]sulfonamide (9c). -- A mixture of 500 mg 1.66 mmol of 2-amino-4-(trichloro-

ethenyl)benzenesulfonamide (10) (1) and 3,600 mg, 30.90 mmol of chloro[^{35}S]sulfonic acid was stirred in an oil bath at 110°C for two hours. After cooling to room temperature and standing overnight the resulting mixture was quenched by pouring into 50 g of water/ice while stirring and cooling in an ice bath. After 30-60 minutes additional stirring at 0°C the solid product was collected and briefly dried (652 mg). This crude material was dissolved in methylene chloride, washed with water, and filtered after treatment with decolorizing carbon. While stirring in a water bath at room temperature ammonia (gas) was passed in for fifteen minutes. TLC indicated complete conversion to (9c). After stirring for an additional hour 100 ml of ethyl acetate was added and the resulting solution was washed with dilute HCl and water, treated with decolorizing carbon, filtered, and evaporated to dryness, to leave a colorless glassy product. Dissolution in ethyl acetate and addition of hexane to turbidity, followed by seeding led to crystallization. Complete separation of product was achieved by addition of more hexane. Collected and dried, this product weighed 361 mg, 0.94 mmol. An additional recrystallization from acetone/water was required to provide material of sufficient purity for tracer use. The final "tracer quality" (9c), 316 mg, 0.82 mmol, represented a yield of 49.1% based on the starting sulfonamide. The specific activity of this product was 74.5 μCi/mg, 28.8 mCi/mmol.

Note: The decay corrected specific activities of the product (9c), 28.8 mCi/mmol, and the chloro[^{35}S]sulfonic acid starting material, 26 mCi/mmol, suggested that essentially only one sulfur-35 enriched atom per molecule was introduced, i.e. little or no equilibration with the sulfonamido group of the starting material (10) had occurred.

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