Synthesis of ^{14}C Isotopic Isomers of Tenidap - A Novel Antiinflammatory Agent

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SUMMARY

Two isotopic isomers of tenidap, a novel antiinflammatory agent, were prepared. Compound **6** (specific activity = 10.24 mCi/mmol), having ^{14}C in the indole ring, was prepared in three steps (52% overall yield) starting from 1H-[^{14}C]indole-2,3-dione. Compound **11** (specific activity = 57.16 mCi/mmol, radiochemical purity = 99.0%), with ^{14}C in the C-3 methylene, was prepared in two steps (66% overall yield) beginning with 2-thiophenecarboxylic-[^{14}C-carbonyl] acid.

Key Words: tenidap, (Z)-5-chloro-2,3-dihydro-3-(hydroxy-2-thienylmethylene)-2-oxo-1H-indole-1-carboxamide sodium salt, (Z)-5-chloro-2,3-dihydro-3-(hydroxy-2-thienylmethylene)-2-oxo-1H-[^{14}C]indole-1-carboxamide sodium salt, (Z)-5-chloro-2,3-dihydro-3-(hydroxy-2-thienyl-[^{14}C]methylene)-2-oxo-1H-indole-1-carboxamide, ^{14}C-tenidap, antiinflammatory.

INTRODUCTION

The *in vitro* preclinical pharmacology profile of tenidap, **1**, shows inhibition of cytokine production (IL-1 and IL-6) (1) and cyclooxygenase (2), thus describing a unique new class of antiinflammatory. These effects are demonstrated in man where tenidap is efficacious in rheumatoid arthritis (3) and osteoarthritis (4) with reductions in serum levels of the acute phase proteins, C-reactive protein and serum amyloid A (5).

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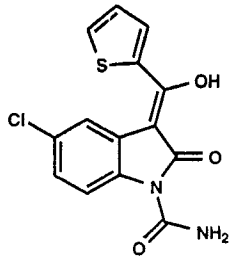


1-Tenidap

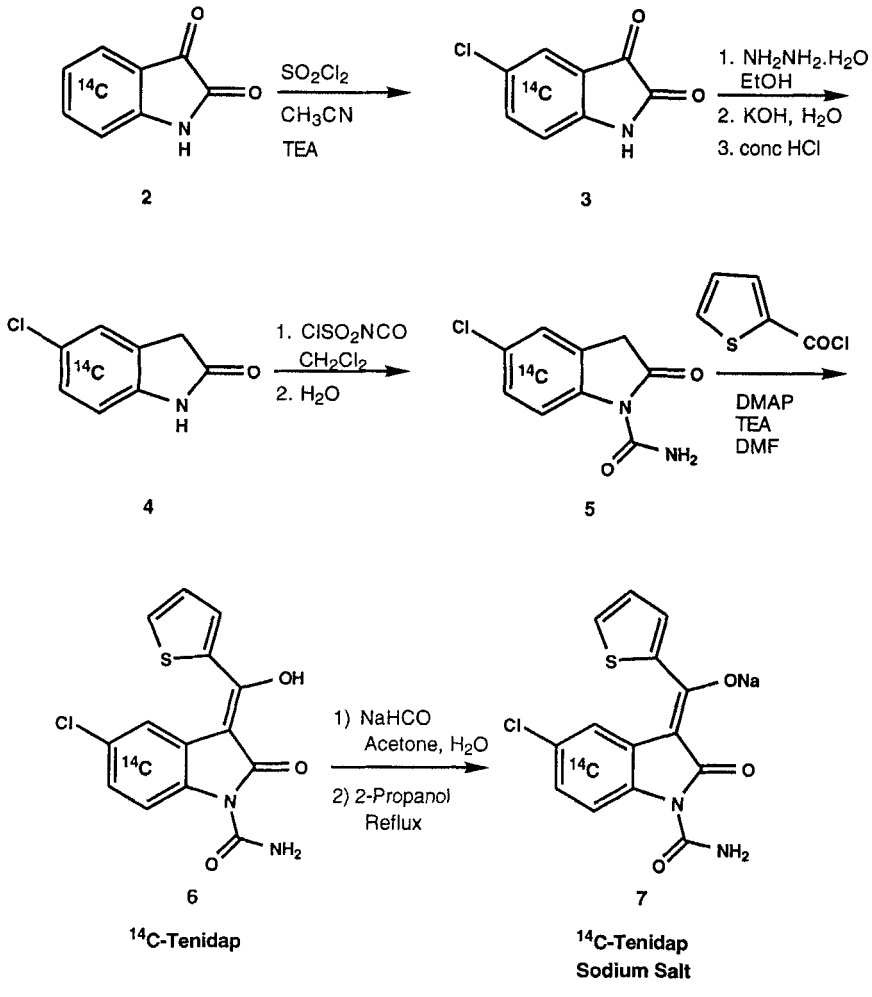
As part of a development program, the metabolic disposition of tenidap was studied in animals and man. Two ^{14}C-radiolabelled isotopic isomers of tenidap were synthesized and used to conduct the desired studies.

RESULTS AND DISCUSSION

^{14}C-Tenidap sodium salt, **1**, with ^{14}C incorporated into the benzo portion of the indole ring was synthesized as outlined in Scheme I. Radiolabelled isatin, **2**, was chlorinated in 75% yield to give regioselectively the 5-chloroisatin **3**. Reduction of the C-3 ketone in **3** was achieved using hydrazine and base to provide intermediate **4** (81% yield). Carbamoylation at N-1 of **4** was efficiently conducted using chlorosulfonylisocynate to give radiolabelled **5** in 97% yield. Compound **5** was coupled with 2-thenoyl chloride using 4-N,N-dimethylaminopyridine as catalyst giving ^{14}C-tenidap, **6** (89% yield). Conversion of **6** to the sodium salt **7** was completed in two steps using sodium bicarbonate first and then dehydration with isopropanol (85% overall from **6**). All intermediates and products were analyzed by thin layer chromatography and shown to co-elute with authentic nonradiolabelled standards. The structure of compound **6** was further confirmed by high resolution mass spectroscopy and its specific activity was determined to be 10.24 mCi/mmol.

To find metabolites resulting from loss of the C-3 substituent on tenidap the ^{14}C-methylene isotopic isomer **11** was prepared as outlined in Scheme II. Using thionyl chloride, 2-thiophenecarboxylic acid, with ^{14}C label in the carboxyl group, was converted into the acid chloride **9**. Coupling of **9** with the indole-1-carboxamide **10** (6) was achieved with 4-N,N-dimethylaminopyridine as catalyst to give ^{14}C-tenidap **11**

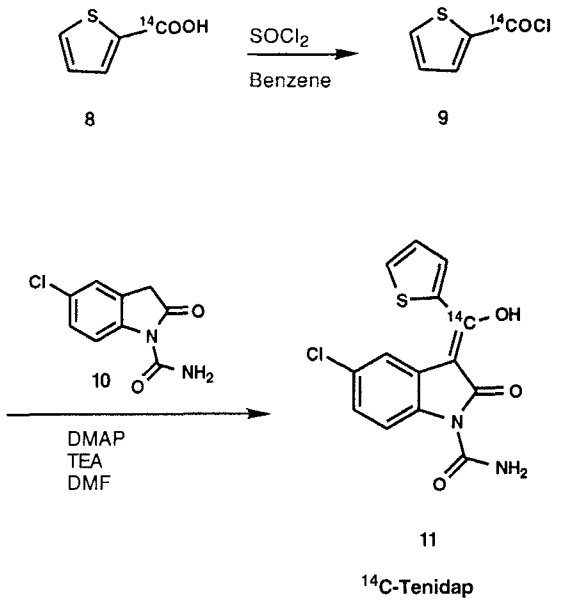
Scheme I



in 77% yield from **8**. Two recrystallizations gave pure **11** in 66% overall yield from **8**. The product was analyzed by thin layer chromatography and shown to co-elute with authentic nonradiolabelled standard. The specific activity of **11** was determined to be 57.16 mCi/mmol.

Compounds **6**, **7** and **11** have been successfully used in animal drug metabolism studies. Drug metabolism studies in man were successfully conducted with ^{14}C-tenidap sodium salt, **7**.

Scheme II



EXPERIMENTAL

The radiolabelled starting material, 1H-[^{14}C]indole-2,3-dione, was purchased from DuPont, NEN Products (97.8% radiochemical purity by thin-layer chromatography and 99% radiochemical purity by HPLC; specific activity=10.80 mCi/mmol). The ^{14}C label was randomly distributed in the benzene ring portion of the 1H-[^{14}C]indole-2,3-dione. The 2-thiophenecarboxylic-[^{14}C-carbonyl] acid, **8**, was purchased from Chemsyn Science Laboratories (99% radiochemical purity by thin-layer chromatography analysis; specific activity = 57 mCi/mmol). High resolution mass spectra (hrms) were obtained on an AEI-MS 30 coupled with a DS50 system. Thin-layer chromatography (tlc) analysis was performed on 0.25 mm silica gel plates (E. Merck, Kieselgel 60F_{254}). All reactions were conducted under a nitrogen atmosphere. Drying *in vacuo* was conducted with a high vacuum pump (0.01-0.5 torr) unless otherwise noted.

5-Chloro-1H-[^{14}C]indole-2,3-dione (**3**). To a 55°C solution of 421 mg (2.86 mmol) of 1H-[^{14}C]indole-2,3-dione, **2**, in 8.6 mL of acetonitrile and 30.1 µL (0.215 mmol) of triethylamine was added in one portion 469 µL (5.85 mmol) of sulfuryl chloride. The reaction was then heated at 65°C for 5 hr. (A precipitate formed starting approximately 5 min after addition of the sulfuryl chloride.) The reaction was cooled to -5°C and let stand for 30 min. The precipitated product was collected by filtration through a sintered glass funnel and washed with 1 mL of ethanol. The collected solid was dried *in vacuo* for 15 hr to give 389 mg (75%) of **3** as a red-orange solid. By tlc analysis (1:1:1 ethyl acetate:hexane:dichloromethane) the product was identical to authentic non-radiolabelled material. No further characterization was performed.

5-Chloro-1,3-dihydro-2H-[^{14}C]indole-2-one (**4**). To a 25°C slurry of 389 mg (2.15 mmol) of 5-chloro-1H-[^{14}C]indole-2,3-dione, **3**, in 4 mL of ethanol was added 136 µL (2.80 mmol) of hydrazine monohydrate and 136 µL (7.55 µmol) of water. The solution was heated at reflux for 2.5 hr to yield a yellow precipitate. The reaction mixture was cooled to 50°C and 388 mg (5.25 mmol) of potassium hydroxide pellets added. The resultant thick brown precipitate was heated at reflux for 3.5 hr yielding a solution. The reaction solution was quenched by cooling to 5°C and adding 0.64 ml (11.5 mmol) of concentrated hydrochloric acid in 4.5 mL of water. The resultant precipitate was stirred 1.5 hr at 25°C, filtered through a sintered glass funnel and the collected solid washed with 1.5 mL of 5°C water. The solid was dried *in vacuo* for 15 hr to give 290 mg (81%) of **4** as an off-white solid. By tlc analysis (1:1:1 ethyl acetate:hexane:dichloromethane) the product was identical to authentic non-radiolabelled material. No further characterization was performed.

5-Chloro-2,3-dihydro-2-oxo-1H-[^{14}C]indole-1-carboxamide (**5**). To a 25°C slurry of 290 mg (1.74 mmol) of 5-chloro-1,3-dihydro-2H-[^{14}C]indole-2-one, **4**, in 5.4 mL of dichloromethane was added 167 µL (1.91 mmol) of chlorosulfonylisocyanate. The resultant partial solution was heated at reflux for 1.5 hr. Water (53 µL) was added and 50% of the dichloromethane was then slowly distilled yielding a new precipitate. Water (2.2 mL) was added and the remainder of the dichloromethane removed by distillation. The resultant thick off-white precipitate was stirred 1.5 hr at 20°C. The reaction precipitate was collected by filtration through a sintered glass funnel and washing with 3.4 mL of water. The resultant solid was dried in vacuo for 18 hr to give

354 mg (97%) of **5** as an off-white solid. By tlc analysis (1:1:1 ethyl acetate:hexane:dichloromethane) the product was identical to authentic non-radiolabelled material. No further characterization was performed.

(Z)-5-Chloro-2,3-dihydro-3-[hydroxy-2-thienylmethylene]-2-oxo-1H-[^{14}C]indole-1-carboxamide, **6**, (^{14}C-Tenidap). To a 0°C mixture of 354 mg (1.69 mmol) of 5-chloro-2,3-dihydro-2-oxo-1H-[^{14}C]indole-1-carboxamide, **5**, and 41.4 mg (0.34 mmol) of 4-N,N-dimethylaminopyridine in 2.58 mL of dimethylformamide was added 0.47 mL (3.39 mmol) of triethylamine followed by 200 µL (1.87 mmol) of 2-thenoyl chloride. The resultant reaction was stirred 3 hr at 25°C and then quenched by addition of 5.2 mL of methanol and warming to 30°C. Concentrated hydrochloric acid (0.41 mL, 7.38 mmol) was added and the resultant mixture cooled to 25°C and stirred for 1 hr. The precipitated solid was filtered through a sintered glass funnel and the collected solid washed with a total of 6 mL of methanol. The resultant solid was dried *in vacuo* for 20 hr to give 466 mg (89%) of **6** as a yellow solid. By tlc analysis (1:1:1 ethyl acetate:acetonitrile:dichloromethane) the product was identical to non-radiolabelled material. HRMS (m/e) 320.0005 (M^+ Calcd for $C_{14}H_9ClN_2O_3S$: 320.0022). Specific Activity (tlc) = 10.24 mCi/mmol.

Purification of (Z)-5-Chloro-2,3-dihydro-3-[hydroxy-2-thienylmethylene]-2-oxo-1H-[^{14}C]indole-1-carboxamide, **6**, (^{14}C-Tenidap). ^{14}C-Tenidap, **6**, (100 mg, 0.31 mmol) was dissolved in 0.7 mL of dimethylacetamide at 75°C in a 15 mL centrifuge tube. The solution was cooled to 65°C, diluted with 1 mL of methanol, cooled to 25°C and allowed to stand for 1 hr. The reaction was centrifuged and the supernatant decanted. Three times, the remaining solid was slurried in methanol, (volumes of 1 mL, 0.5 mL and 3 mL respectively), centrifuged and the supernatant decanted. The final remaining solid was dried *in vacuo* at 69°C for 30 min and then 15 hr at 25°C to give 95.6 mg (95.6%) of **6** as a yellow solid. ^{14}C-Tenidap, **6**, (79.1 mg, 0.247 mmol) was mixed with 0.9 mL of acetic acid in a 15 mL centrifuge tube and refluxed for 4 hr. After cooling to 25°C the reaction was centrifuged and the supernatant decanted. The remaining solid was slurried in 0.4 mL of acetic acid, then centrifuged and the supernatant decanted. The remaining solid was dried *in vacuo* at 60°C for 15 hr to give 75.6 mg (95.6%) of **6** as a yellow solid.

(Z)-5-Chloro-2,3-dihydro-3-[hydroxy-2-thienylmethylene]-2-oxo-1H-[^{14}C]indole-1-carboxamide sodium salt, (7, ^{14}C-Tenidap sodium salt). A mixture of 9.54 g (29.8 mmol) of tenidap, **1**, 56 mg (0.175 mmol) of ^{14}C-tenidap, **6**, (from the above purification procedure) and 2.64 g (31.4 mmol) of sodium hydrogen carbonate in 76.6 mL of acetone was heated to 50°C. While maintaining the reaction at 50°C, 38.9 mL of water was added over 30 min. The reaction was filtered warm through celite and the celite pad washed further with 5 mL of acetone. The filtrate was evaporated under vacuum to a thick slurry which was filtered at 20°C and the collected solid washed with 9.5 mL of water. The solid obtained was dried *in vacuo* at 60°C for 15 hr to give 10.2 g (94.7%) of ^{14}C-tenidap sodium salt monohydrate as a yellow solid. A mixture of the monohydrate (10.2 g, 28.3 mmol) and 51.1 mL of 2-propanol was heated and stirred at reflux for 5 hr. The mixture was allowed to cool to 25°C, filtered and the collected solid was washed with 15 mL 2-propanol. The solid obtained was dried *in vacuo* at 60°C for 15 hr to give 9.56 g (98.5%) **7** as a yellow solid.

(Z)-5-Chloro-2,3-dihydro-3-[hydroxy-2-thienyl-[^{14}C]methylene]-2-oxo-1H-indole-1-carboxamide, (**11**, ^{14}C-Methylene tenidap). To 112 mg (0.874 mmol) of 2-thiophenecarboxylic-[^{14}C-carbonyl] acid, **8**, in 1 mL of benzene was added 80 µL (1.10 mmol) of thionyl chloride. The reaction was then heated at reflux for 4 hr followed by cooling to 25°C. The just formed solution of 2-thiophene [^{14}C]-carbonyl chloride was transferred to a 50mL plastic centrifuge tube with three 1 mL washings of benzene. The benzene solvent was then evaporated under a stream of nitrogen gas at 25°C. With warming, a solution was prepared of 220 mg (1.05 mmol) of 5-chloro-2,3-dihydro-2-oxo-1H-indole-1-carboxamide, **10**, (6) and 213 mg (1.74 mmol) of 4-N,N-dimethylaminopyridine in 2.3 mL of dimethylformamide followed by cooling of the solution to 0°C. This cold solution was added to the above centrifuge tube containing the evaporated acid chloride solution and the reaction stirred 10 min at 0°C and 30 min at 25°C. The reaction was quenched by cooling to 0°C followed by addition of 9 mL of 1N hydrochloric acid. The resultant mixture was stirred at 0-25°C for 10 min and then diluted with 7 mL of water. The mixture was centrifuged and the supernatant decanted. The remaining solid was slurried with 10 mL water, centrifuged and the supernatant decanted. This remaining solid was slurried with 10 mL of 50% water-methanol, centrifuged and the supernatant decanted. The final remaining solid was

dried 15 hr in a dessicator over anhydrous calcium sulfate to yield crude ^{14}C-tenidap, **11**. To the crude **11** was added a solution of 80 µL (1.33 mmol) of ethanolamine in 6 mL of methanol. The resultant solution was diluted with 16 mL of methanol and mixed. This mixture was centrifuged and the supernatant decanted to a 50 mL plastic centrifuge tube. The decanted solution was acidified with 12 mL of 1N hydrochloric acid and mixed. This mixture was centrifuged and the supernatant decanted. The remaining solid was washed, centrifuged and decanted three times (twice 10 mL water, once with 10 mL 50% water-methanol). The solid was dried 72 hr *in vacuo* at 60°C to give 216 mg (77%) of **11** as a yellow solid. This recrystallization procedure was repeated twice on the 216 mg sample of **11** and each time the remaining solid was washed with 10 mL of isopropyl ether. The final product was dried 48 hr at 25°C *in vacuo* to give 185 mg (66%) of **11** as a yellow solid. Radiochemical Purity (tlc) = 99.0%. Chemical Purity (tlc) = 99.9%. Specific Activity (tlc) = 57.16 mCi/mmol.

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