SYNTHESIS OF TWO ^{14}C-LABELED FORMS OF PRINOMIDE, A POTENTIAL ANTI-INFLAMMATORY COMPOUND AND ITS HYDROXY METABOLITE, LABELED WITH ^{14}C.

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SUMMARY

The synthesis of two ^{14}C-labeled forms of prinomide, compounds 7 and 10, and the synthesis of a ^{14}C-labeled form of its hydroxy metabolite are described. Compound 7 was synthesized by the reaction of phenyl isocyanate and 1-methyl-β-oxo-pyrrole-2-propanenitrile-carbonyl-^{14}C (6), which was synthesized in three steps from 1-methylpyrrole and $^{14}CO_2$. Cpmpound 10 was synthesized by the reaction of 8, the unlabeled form of the above ketonitrile, and ring-labeled phenyl isocyanate (9), which was synthesized in three steps from benzene-^{14}C. The ^{14}C-labeled form of the hydroxy metabolite 14 was synthesized by reaction of 4-methoxyphenyl isocyanate and the ^{14}CN-labeled ketonitrile 12, followed by demethylation with boron tribromide. Labeled ketonitrile 12 was synthesized from 1-methylpyrrole, by reaction with chloroacetonitrile, followed by replacement of the chlorine atom by a ^{14}CN group.

KEY WORDS: Prinomide, hydroxy metabolite of prinomide, phenyl-$^{14}C_6$-isocyanate.

INTRODUCTION

The search for aspirin-like anti-inflammatory drugs for the treatment of arthritis and other inflammatory conditions has led to the discovery of several new drugs in recent years which are designated as NSAIDS (non-steroidal anti-inflammatory drugs). Prinomide, 3-hydroxy-3-(1-methyl-1H-pyrrol-2-yl)-2-(phenylcarbamoyl)-2-propenenitrile (1), which has recently been discovered in our research department (1), is a potential NSAID, currently under clinical trial. Preliminary data indicate that it may also have therapeutic properties of a disease-modifying agent.

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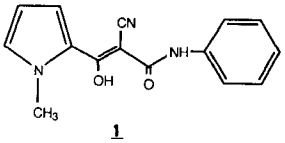
Two ^{14}C-labeled forms of this compound, 7 and 10 were synthesized (Scheme 1) for pharmacokinetic and metabolism studies. Compound 10 was needed specifically to investigate whether the anilide bond undergoes fission to produce aniline or any of its transformation products. Some of the metabolism studies using compound 7 have been published by Egger et al (2). These studies showed that the cyano group was not lost by metabolism and a major metabolite, compound 2, was formed by aromatic hydroxylation at the para position of the benzene ring. We synthesized a ^{14}C-labeled form of this metabolite, compound 14 (Scheme 2), for further studies.



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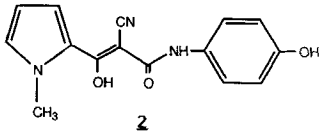
PRINOMIDE



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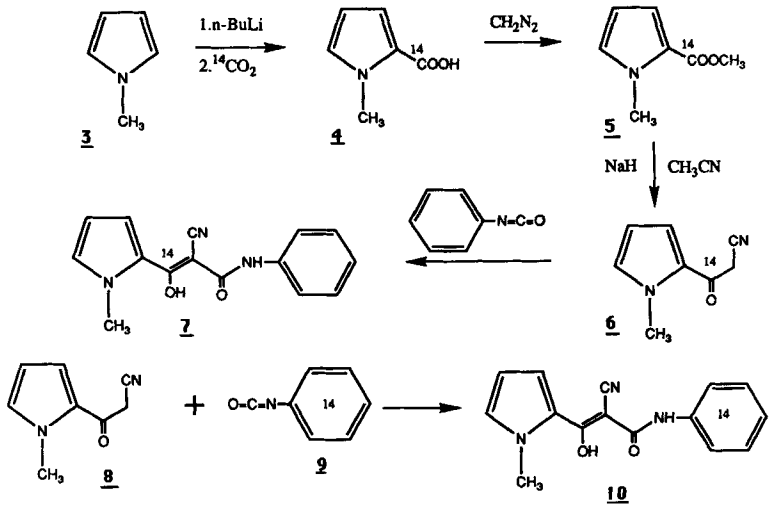
METHODS AND RESULTS

The synthetic routes for the preparations of 7 and 10 are shown in Scheme 1. The starting material, 1-methylpyrrole (3) was treated with n-butyllithium at -70°C and the lithium complex was carbonated with $^{14}CO_2$ to produce 1-methyl-2-pyrrolecarboxylic acid (4). The corresponding methyl ester 5, prepared by reaction of 4 with diazomethane was condensed with acetonitrile to give the ketonitrile 6. Compound 6, which has a reactive methylene group, readily reacted with phenyl isocyanate to yield compound 7.

Compound 10 was synthesized by reacting compound 8, the unlabeled form of the above ketonitrile, with ring-labeled phenyl isocyanate (9). The unlabeled ketonitrile 8 was synthesized from 1-methylpyrrole (3) by the above method using CO_2, instead of $^{14}CO_2$, for carbonation. Ring-labeled phenyl isocyanate (9) was synthesized from benzene-^{14}C via nitrobenzene. Catalytic hydrogenation of nitrobenzene in the presence of hydrogen chloride gave aniline hydrochloride, which was then reacted with phosgene at 110°C to give phenyl isocyanate (9).

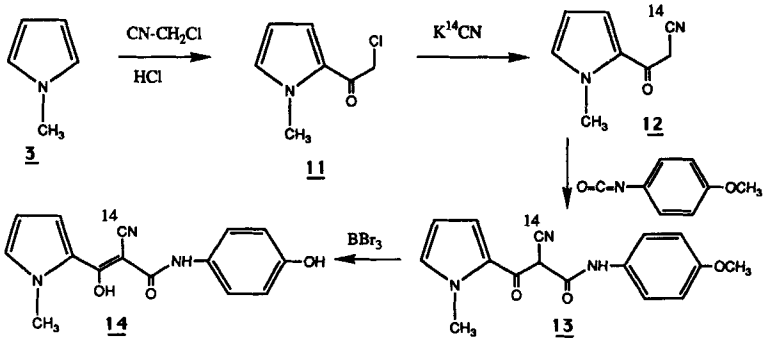
For the synthesis of the hydroxy metabolite 14, (Scheme 2), 1-methylpyrrole 3 was reacted with chloroacetonitrile in the presence of

Scheme 1



hydrogen chloride to yield chloroacetyl compound 11. The chlorine atom of 11 was then replaced by a labeled cyano (^{14}CN) group by reaction of 11 with $K^{14}CN$, catalyzed by 18-crown-6 ether, to give the ketonitrile 12. The yield of 12 in the uncatalyzed reaction was very poor. Compound 12 was then treated with 4-methoxyphenyl isocyanate to yield the methoxy compound 13, which was demethylated by reaction with a large excess of boron tribromide to give the hydroxy compound 14.

Scheme 2



EXPERIMENTAL

Melting points are uncorrected. Thin layer chromatographic (TLC) was carried our on silica gel 60F 254 plates of 0.5 mm thickness. $Ba^{14}CO_3$ and benzene-$^{14}C_6$ were purchased from NEN DuPont Co.

1-Methylpyrrole-2-carboxylic acid-carboxy-^{14}C (4). A solution of 1.13 g of 1-methylpyrrole in 15 ml of tetrahydrofuran was cooled to -70°C, and to it was added with stirring 10 ml of 1.5M n-butyllithium in hexane. After 15 min, the cooling bath was removed. The mixture was brought to room temperature and stirring was continued for 2 hr. The reaction flask was connected to a vacuum line, and $^{14}CO_2$, generated from 2.37 g (12 mmol) of $Ba^{14}CO_3$ (sp. activity, 5 mCi/mmol; total activity, 60 mCi) was absorbed in the solution, cooled to 0°C. The mixture was stirred for 2 hr at 0°-5°C, then acidified by adding 2N HCl solution. Unreacted $^{14}CO_2$ was removed by passing a stream of nitrogen, and the mixture was extracted twice with dichloromethane. The dichloromethane solution was dried with $MgSO_4$ and then evaporated to dryness to yield 1.02 g of a solid material; m.p., 135-136°C, undepressed on admixture with authentic unlabeled acid (Aldrich).

1-Methyl-β-oxo-pyrrole-2-propanenitrile-carbonyl-^{14}C (6). A solution of 1.01 g of the above acid 4 in 20 ml of ether was cooled to 0°C, and then treated with an ether solution of diazomethane at 0°-5°C. After one hour, ether was removed to give the methyl ester 5 as an oil. The oil was mixed with 1.5 ml of dimethylformamide and 0.67 g of sodium hydride, dispersed in oil, and the mixture was stirred. To the above mixture was then added 1.8 ml of acetonitrile, and the mixture was heated at 80°C for 2 hr. Water was added to the mixture which was then extracted with ether. The ether extract was discarded. The aqueous part was acidified and then extracted with dichloromethane. The dichloromethane extract was dried with $MgSO_4$ and evaporated to dryness. The residue was then chromatographed on a column of silica gel. The radioactive fraction eluted with toluene-ethyl acetate (3:1) was evaporated to give a solid; m.p. 106°-108°C. TLC in toluene-ethyl acetate (3:1) showed only one UV-visible spot, identical to that of unlabeled compound 8, prepared by the same method from 1-methylpyrrole carboxylic acid (Aldrich).

3-Hydroxy-3-(1-methyl-1H-pyrrol-2-yl)-2-(phenylcarbamoyl)-2-propenenitrile-3-^{14}C (7). ([^{14}C]CGS 10787). To a solution of 424 mg of the above solid 6 in 8 ml of toluene, was added 0.5 ml of triethylamine and 0.35 ml of

phenylisocyanate, and the mixture was stirred overnight at room temperature. Toluene was removed from the mixture by evaporation under reduced pressure. The residue was dissolved in 0.5 ml of methanol and the solution was acidified with 2N HCl. The mixture was extracted with dichloromethane, and the extract was dried with $MgSO_4$ and then evaporated to dryness. The residue was crystallized from isopropanol to yield 750 mg of a pale yellow solid, m/z, 267 (M+); m.p. 179-182°C, same as that of authentic CGS 10787; specific activity, 4.8 mCi/mmol; total activity, 13.5 mCi (22.5% overall yield based on $Ba^{14}CO_3$). The above solid, which is the enolic compound 7, was then converted into a triethanolamine salt (m.p., 121-122°C) for animal studies.

Phenyl-$^{14}C_6$ isocyanate (9). A mixture of 2.4 ml of concentrated nitric acid and 2.8 ml of concentrated sulfuric acid was cooled to 0°C. A solution of 700 mg of benzene-$^{14}C_6$ (15 mCi, sp. activity, 1.67 mCi/mmol) in 2 ml of dichloromethane was added dropwise to the acid mixture, cooled to 0°C. After stirring for 30 min at 0°C, ice-water was added to the mixture which was then extracted two times with dichloromethane. The extract was washed with 2N NaOH solution and water. After drying with $MgSO_4$, the extract was evaporated to give a yellow oil of nitrobenzene; TLC in ether-petroleum ether (1:9) showed a single UV-visible spot identical to that of nitrobenzene. The yellow oil was dissolved in 15 ml of ethanol containing 7% hydrogen chloride. To this solution was then added 800 mg of 10% palladium-on-charcoal, and the mixture was shaken in an atmosphere of hydrogen at 50 psi for 1 hr. The mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure to give 900 mg of aniline hydrochloride. Last trace of ethanol was removed from the solid by co-distillation with toluene.

To the above solid was then added 50 ml of a 20% solution of phosgene in toluene, and the mixture was heated at 110°C for 1 hr. Toluene was removed by distillation under reduced pressure in a rotary evaporator. To the residue was again added 50 ml of the above phosgene solution, and the mixture was heated at 110°C for 1 hr, when all the solid went into solution by reaction with phosgene. Toluene was removed in a rotary evaporator by distillation under reduced pressure to give 600 mg of phenyl isocyanate (9),

which was used in the next step.

3-Hydroxy-3-(1-methyl-1H-pyrrol-2-yl)-2-(phenyl-$^{14}C_6$-carbamoyl)-2-propenenitrile (10). The above oil 9 was dissolved in 25 ml of toluene, and to the solution was added 750 mg of unlabeled compound 8 and 1 ml of triethylamine. The mixture was stirred overnight at room temperature and the reaction product was isolated as described above for the preparation of 7. The crude reaction product was dissolved in 2N NaOH solution and some insoluble material was removed by filtration. The filtrate, which showed no impurity by TLC in ethyl acetate, was acidified with 2N HCl. The precipitated solid was filtered and dried in vacuo at 60°C for 24 hr; m.p., 179-182°C, same as that of authentic unlabeled compound; yield 900 mg; sp. activity, 1.65 mCi/mmol; total activity, 5.58 mCi.

2-Chloro-1-(1-methyl-1H-pyrrol-2-yl)ethanone (11). To a solution of 10.0 g of chloroacetonitrile in 50 ml of ether, saturated with hydrogen chloride, was added dropwise 11.7 ml of 1-methylpyrrole. After the initial exothermic reaction subsided, the mixture was stirred at room temperature for 30 min. The ether layer was decanted off, and the oily residue was washed twice with ether. The residue was treated with water and stirred for 10 min. The aqueous mixture was then extracted three times with ethyl acetate. The ethyl acetate extract was dried with $MgSO_4$ and evaporated to dryness. The residue on trituration with pertoleum ether turned to a gray solid (8.49 g).

1-Methyl-β-oxo-2-propanenitrile-cyano-^{14}C (12). A mixture of 13 mg of $K^{14}CN$ (20 mCi, sp. activity, 5 mCi/mmol) and 13 mg of unlabeled KCN was added to 15 ml of acetonitrile containing 100 mg of 18-crown-6-ether. To the above mixture was then added 632 mg of compound 12, and the mixture was stirred at room temperature for 2 days. Acetonitrile was removed by evaporation under reduced pressure, and the residue was treated with 15 ml of water. The mixture was extracted three times with ethyl acetate. The ethyl acetate extract was dried with $MgSO_4$ and evaporated to dryness. The residue was purified by chromatography on a column of silica gel. The radioactive fraction eluted with petroleum ether/ethyl acetate (90/10, v/v) was evaporated to give 457 mg of a solid, which had an infra red band at 2220

cm^{-1} due to the nitrile group of 12; m.p., 108-109°C, same as that of an authentic unlabeled compound.

3-Hydroxy-2-(4-methoxyphenylcarbamoyl)-3-(1-methyl-1H-pyrrol-2-yl)-2-propenenitrile-cyano-^{14}C (13). To a solution of 450 mg of the above compound 12 in 5 ml of toluene was added 450 mg of 4-methoxyphenyl isocyanate and 0.5 ml of triethylamine. The mixture was stirred overnight at room temperature and then quenched with addition of 20 ml of water. The toluene layer was separated and discarded. The aqueous part was acidified by adding 2N HCl. The precipitated solid was filtered, washed with water, and then crystallized from ethanol. The crystallized material was filtered and dried to yield 750 mg of 13, m.p., 191-193°C, same as that of authentic unlabeled compound. TLC in chloroform/methanol (95/5, v/v) showed only one UV-visible sopt.

3-Hydroxy2-(4-hydroxyphenylcarbamoyl)-3-(1-methyl-1H-pyrrol-2-yl)-2-propene-nitrile-cyano-^{14}C (14). To a solution of 500 mg of the above compound 13 in 10 ml of dichloromethane was added 6 ml of 1M BBr_3 solution in dichloromethane. The mixture was stirred at room temperature for 72 hr and then evaporated to dryness. The residue was treated with water and filtered. The solid was treated with 0.1N NaOH solution and filtered. The filtrate was extracted with ethyl acetate and the extract was discarded. The aqueous part was acidified with 1N HCl solution. The precipitated solid was filtered, washed with water and dried. It was then crystallized twice from ethanol to yield 240 mg of compound 14; m.p., 188-190°C, same as that of authentic unlabeled compound; m/z, 283 (M+); sp. activity, 4.81 mCi/mmol; total activity, 4.28 mCi.

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